Epidemiologic aspects of mass deworming in Nigerian schools

Dissertation for the Degree of Doctor rerum medicinae (Dr. rer. med.) at the Faculty of Medicine, University of Leipzig, Germany

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Abstract

With the current trends in integrated management of childhood diseases in developing countries, it is important to resolve the controversies of coinfections between helminths and malaria, and properly evaluate the epidemiology of diarrhegenic parasites with molecular study, which sometimes cause overlapping infections. Again, the major challenge facing the global success of mass deworming initiative aimed at controlling helminths is lack of sustainability due to limited donor funds. We therefore decided to evaluate the effectiveness of a school based deworming program using only the school teachers without spending money on training and logistics. Demographic information, height and weight were measured and stool samples were collected from pupils in a semi-rural area of Nigeria during the initial visit by the study team. Malaria cases were recorded over a 3 month malaria transmission period prior to stool sampling.Four hundred and seventy six (33%) of the study population was infected with one Soil transmitted helminth (STH) or the other, especially with *Askaris lumbricoides* (26.0%) and *Hookworm* (8.4%). We found a negative association between malaria and STH in this community. Helminth infection rate of 18.3% was observed in children with malaria compared to 34.4% in controls. We also found a high carriage rate of *Giardia* (37.2%), low Cryptosporidium (1%) and no *E. histolytica infection* contrary to previous studies that were based on traditional diagnostic techniques. There was 7.9% reduction in the number children with low weight-for-age in the helminth infected children at 6 months after mass deworming, the number of uninfected children with low weight-for-age also reduced by 3.2%. There was also a reduction in the number of children with more than 25% absenteeism among both helminth infected (13.9%) as well as uninfected (7.2%). The association between malaria and STH in our study calls for the need for integrated approach to health problem in Africa instead of the common vertical campaigns. Results from our molecular study also shows the need to strengthen collaborations between researchers from developed and developing countries to be able to map out the true epidemiology of these parasites and hopefully produce novel, inexpensive diagnostics that circumvent the need for advance technological infrastructure

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Abbreviations

AIDS	Acquired immunodeficiency syndrome
BMI	Body mass index
DAAD	Deutscher Akademischer Austauschdienst
ELISA	Enzyme-Linked Immunosorbent Assay
FMC.Path (Nig)	Fellow Medical College of Pathologists (Nigeria)
GBD	Global Burden of Diseases
HIV	Human immunodeficiency virus
IMCI	Integrated management of childhood illness
IPTi	Intermittent preventive treatment in infants
IRS	Indoor Residual Spraying
IVM	Integrated Vector Management
ITN	Insecticide treated net
LLIN	Long-lasting Insecticidal Nets
MDGs	Millennium development Goals
NTDs	Neglected tropical diseases
ORS	Oral rehydration salts
PC	Preventive chemotherapy
PCR	Polymerase Chain Reaction
SAC	School Aged Children
SP	Sulfadoxine/ pyrimethamine

ST-ETEC	Heat-stable Enterotoxigenic *E coli*
STH	Soil Transmitted Helminths
WAEC	West African Examination Council
WHO	World Health Organization
WMR	World Malaria Report
WSH	Water, Sanitation and Hygiene**.**

1.0 Introduction

The World Health Organization projected that infectious diseases including malaria, helminths and diarrhea agents will be the primary causes of death in African region by the year 2030. [1] This is not surprising because diarrhea diseases and malaria have consistently maintained their positions as the 2nd and the 3rd on the list of the top 10 global causes of Non-neonatal pediatric death between year 2000 and 2010 following pneumonia. [2] (Figure 1).

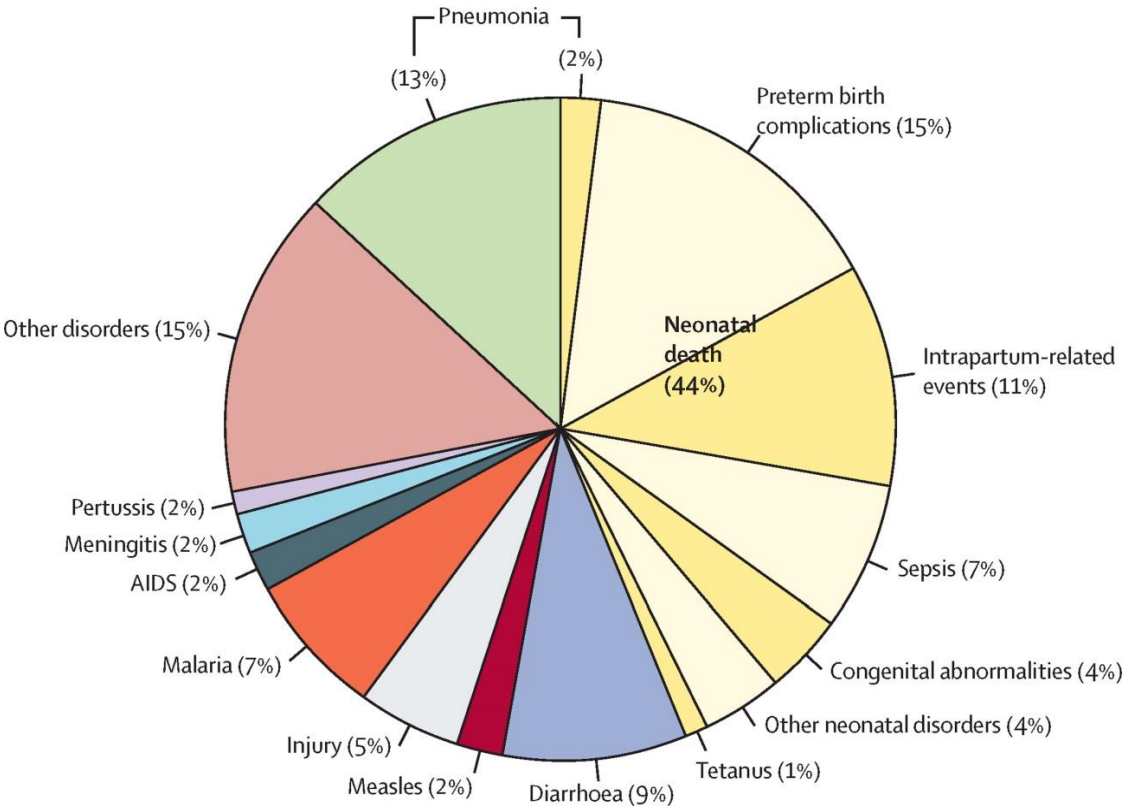

Figure 1. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis.

Only 5 countries of the world including India, Nigeria, Democratic republic of Congo, Pakistan and China accounted for half of the global deaths from infections in the year 2010, while Nigeria was said to have recorded the largest number of malaria deaths [2]. About 30% of the global cases of STH are in African Region of the WHO with more than 40% of them in

only 3 countries (the Democratic Republic of the Congo, Ethiopia and Nigeria) where Nigeria leads with 21%. [3]

1.1 Malaria

Malaria is caused by five species of the parasite belonging to the genus *Plasmodium*. *P. falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi*. The first 4 are human malaria species, which are spread from one person to another by female mosquitoes of the genus *Anopheles*. *P. knowlesi* causes malaria among monkeys in forested areas of South-East Asia but in recent years has been reportedly responsible for human malaria cases.[4] *P. falciparum* is the predominant specie in African where it is responsible for most of the malaria deaths, *P. vivax* has a wider geographic distribution because it can develop in the mosquito vector at lower temperatures and higher altitudes. Although *P. vivax* can occur throughout Africa, the risk of infection is relatively lower because majority of black Africans, particularly West Africans, lack the Duffy gene, which produces the Duffy protein necessary for *P. vivax* attachment to erythrocytes. [5] According to the 2014 World malaria report (WMR), 198 million cases of malaria occurred globally in 2013 which led to about 584 000 deaths. But then, it was believed that the figures represent a decrease in malaria incidence and mortality rates by 30% and 47% respectively since 2000. Malaria is most rampant in the WHO African Region (Figure 2), where an estimated 90% of all malaria deaths occur, and in children below the age of 5 years, who account for 78% of all deaths.[5]

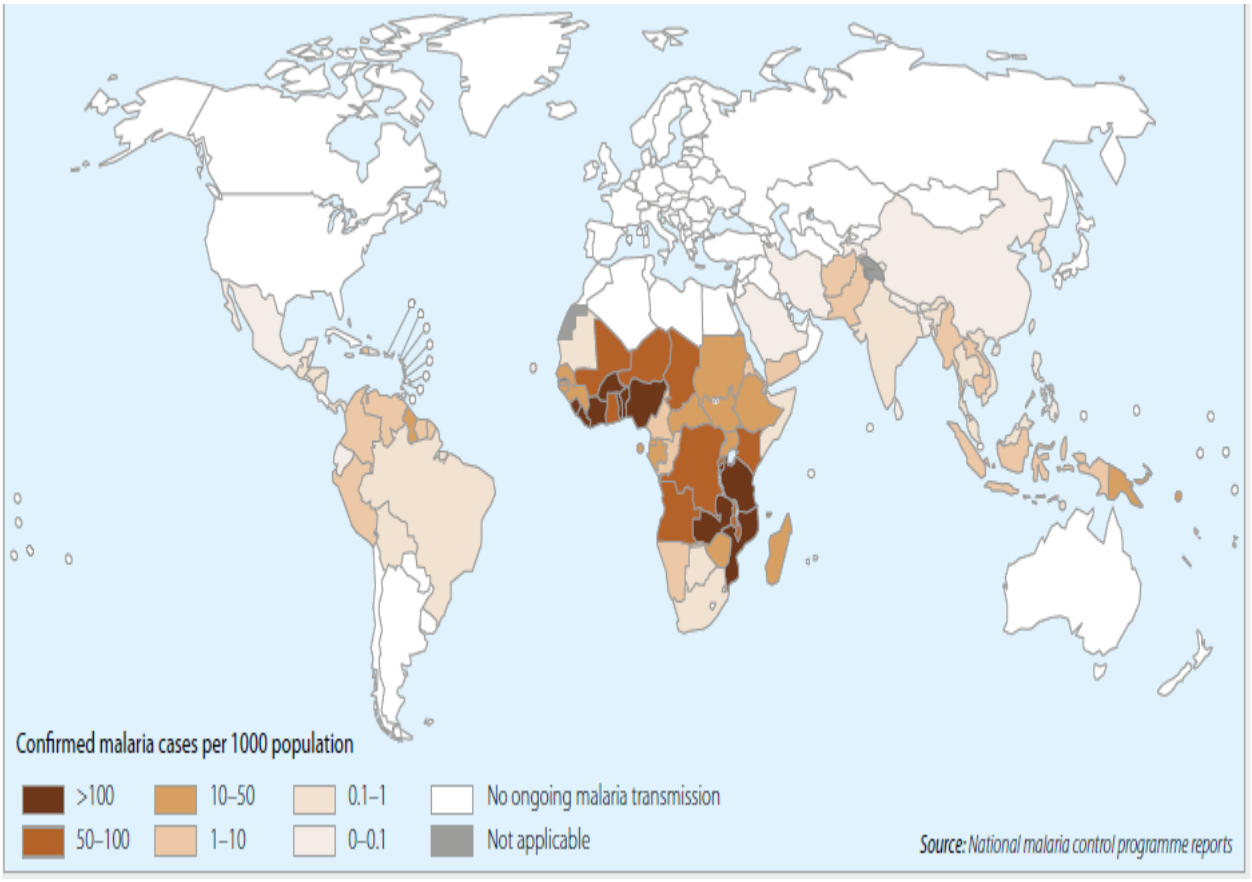

Figure 2. Countries with ongoing transmission of malaria, 2013.

Source: WHO, World malaria report, 2014.

In Africa and in Nigeria, the global epidemiology of the causes of childhood mortality seems to be rearranged. From the 3rd position in global ranking, malaria becomes the 2nd killer disease in Africa by displacing diarrhea. Eventually in Nigeria, malaria comes 1stonthe list by relegating pneumonia and diarrhea to the distance 3rd and 6th respectively on the list of top ten causes of Years of Life Lost (YLL) in Western Sub-Saharan Africa.[6] For instance, the WHO 2010 report on country specific causes of death, indicated that from the 700 000 children that died in Nigeria before their fifth birthday, malaria (20%) was reported to have killed more than pneumonia (17%) and diarrhea (11%).[7]

The prominent status of malaria over other childhood killer diseases in West Africa might not be the accurate reality of the situation on ground because of the increased reporting caused by improved diagnosis due to the upsurge in the deployment of rapid diagnostic kit. It was

reported that in 2013, the number of rapid diagnostic tests (RDTs) procured globally increased from 46 million in 2008 to 319 million in 2013.[8] This has helped to move the diagnosis of malaria from signs and symptoms based to what is now backed by laboratory evidence in many cases. Other major childhood killer diseases, especially diarrhea, did not have similar diagnostic advantage and still depended a lot on signs and symptoms with empirical treatment. This is probably because of the diversity of possible diarrhea agents compared to malaria.

1.1.1 Malaria Intervention Package

The core interventions for malaria control in Nigeria involve the following measures include[9]:-

Prevention of malaria transmission through vector control as part of an Integrated Vector Management strategy (IVM). This includes the use of Long-lasting Insecticidal Nets (LLIN) as well as Indoor Residual Spraying (IRS):- Free distribution of LLIN was adopted by Nigerian government in 2001[(WMR 2014).5] Recent national survey showed that the ownership of ITN increased in Nigeria from 8% in 2008 to 50% in 2013 but the proportion of <5 children sleeping under ITN only increased from 6% to 17% over the same period. IRS on the other hand is not operational in Nigeria since its adoption in 2007.[10]

Prompt diagnosis and adequate treatment of clinical cases at all levels and in all sectors of health care with special attention to management of severe malaria cases: - This package was also was also not maximally implemented in Nigeria, for instance the proportion of under 5 children with febrile illness tested for malaria was less than 5% in 2010 and increased slowly to about 10% as in 2013. Artemisinin Based Combination Therapy (ACT) as the percentage of all antimalarial received by <5 children also increased from about 5% in 2007 to about 20% in 2013. This data represent information from the public health sector, there is poor data

collection from private health sector from where significant proportion of Nigerians receive health care services where the implementation may even be lower.[5]

Prevention and treatment of malaria in pregnancy through the measures above and additional implementation of intermittent preventive treatment in pregnancy:-The 2013 report of the Demographic and Health Survey revealed that the implementation of this particular intervention package is also poor. For instance, the proportion of women who received at least 2 doses of sulfadoxine-pyrimethamine during their last pregnancy at an antenatal care visit was 5% in 2008 and only increased to 15% in 2013.[10] Some of the factors responsible for the poor uptake of IPTs in Nigeria include disapproval of spouses, perceptions of unfriendly attitudes of health workers and long waits. [11]

1.2 Soil-transmitted helminths (STH)

Soil-transmitted helminth (STH) is a term referring to a group of parasitic diseases caused by round worms that are transmitted to humans by fecally contaminated soil. The epidemiology of intestinal helminths is an area that needs a continuous review not only because of the role these parasites play in the co-morbidity of other disease conditions, including diarrhea and malaria, but also because they constitute a great disease burden even when they stand alone. The STH of major concern to humans are *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* and *Ancylostoma duodenale* which are more rampant in the tropical regions where moisture and temperature is mostly favorable, and importantly water supply and sanitation is inadequate leading people to defecate openly. (Figure 3).[3] More than 2 billion people are infected with these parasites where heavy intensity may impair physical growth and cause poor cognitive development, micronutrient deficiencies and anemia leading to poor school performance and absenteeism in children. Heavy infestation also has direct effect on the gastrointestinal channel of transmission by serving as a reservoir for soil and water

contamination. Reduced work productivity in adults and adverse pregnancy outcomes have also been attributed to STH infections.[3]

1.2.1 STH and Preventive chemotherapy (PC)

In 2001, WHO recommended the integration of STH control into existing primary health-care. [12] It was also recommended that STH drugs should be packaged as an integrated preventive chemotherapy targeting other neglected tropical diseases (NTDs) such as lymphatic filariasis, onchocerciasis and schistosomiasis. The strategy recommended by WHO[13] to control morbidity from STH (defined as the elimination of infections of moderate and high intensity) involves the periodic administration of preventive chemotherapy (PC) tablets with single dose of albendazole (400 mg) or mebendazole (500 mg) to at risk population especially preschool-aged and school-age children. The frequency of PC usually depends on the intensity of infection. In communities with high intensity with prevalence greater that 50%, twice a year mass deworming is recommended while low intensity communities with prevalence between 20-50% should be dewormed once a year. Members of a community with low infection intensity could be treated on case-by-case basis.[13] Despite the huge commitment of the WHO and donor partners to school-based PC program, it has remained difficult to sustain in most countries because donor funds are dwindling. Yet majority of the funds budgeted is spent on training and logistics. The programs also become more expensive because it required the collaboration of many agencies such as finance, education, health, environment and many more. We therefore decided to evaluate the effectiveness of a school based deworming program using only the school teachers, and without formal training

1.2.2 Aim and rationale of PC

The aim of preventive chemotherapy is to guard against the widespread morbidity that goes with helminthiasis as well as sustained reduction in worm transmission.[14] PC involves mass administration of good quality drug or combination of drugs, free of charge, with full coverage of all at-risk members in an endemic community rather than trying to identify only the infected individuals. One major advantage of the drugs administered is their excellent safety profile, even in pregnancy.[14] In addition to averting the overt morbidity of STH such as intestinal obstruction, growth retardation and micronutrient deficiencies, PC has been found to also prevent subclinical organ damage along with subtle and long term morbidity such as increased school absenteeism, impaired cognitive performance, reduced worker productivity and lowered self-esteem that occur later in life. [15,16]

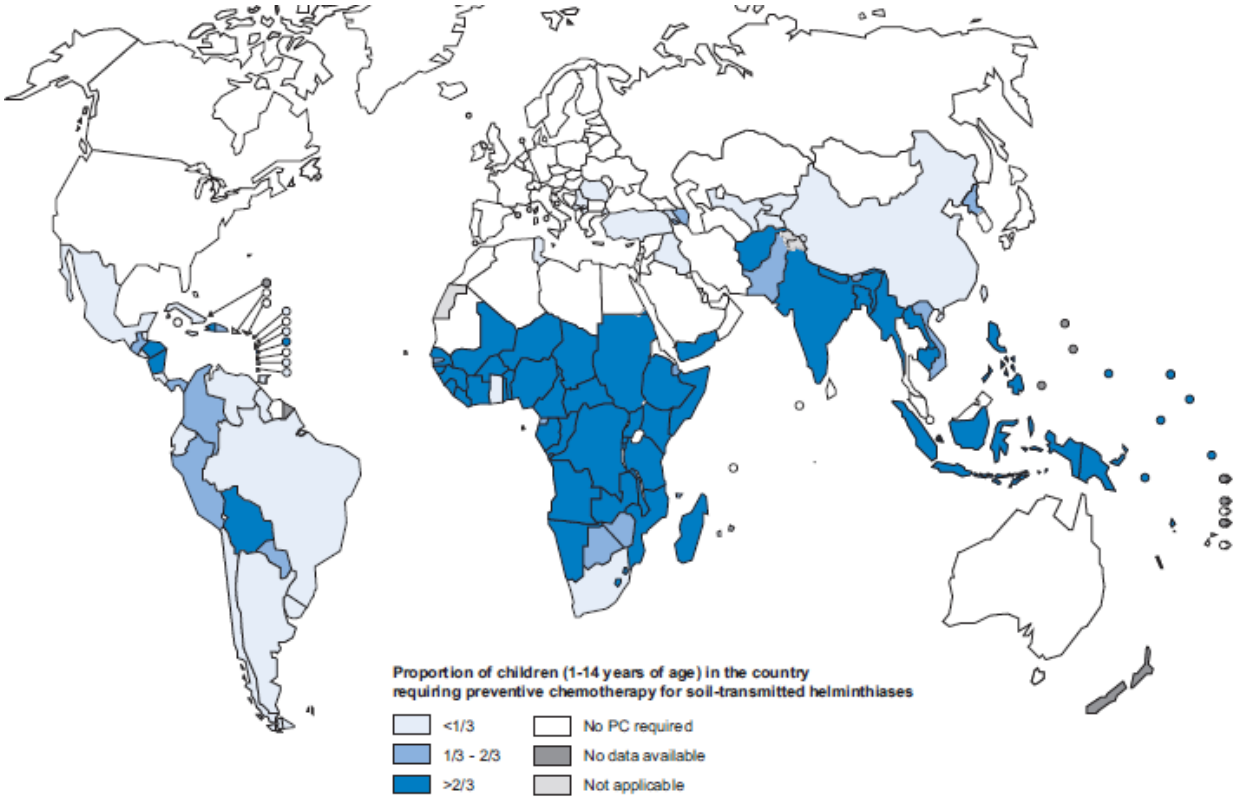

Figure 3. Proportion of children requiring preventive chemotherapy (PC) for STH globally by country in 2009. Source:- WHO STH report, 2001-2010

Nigeria (21%) has the largest number of children requiring preventive chemotherapy for soil-transmitted helminthiases in Africa followed by Ethiopia (12%) and Democratic Republic of the Congo 10%[3] (Figure 4). Therefore, the country qualifies as a priority area where continuous research and impact assessment of PC should be routinely and continuously carried out.

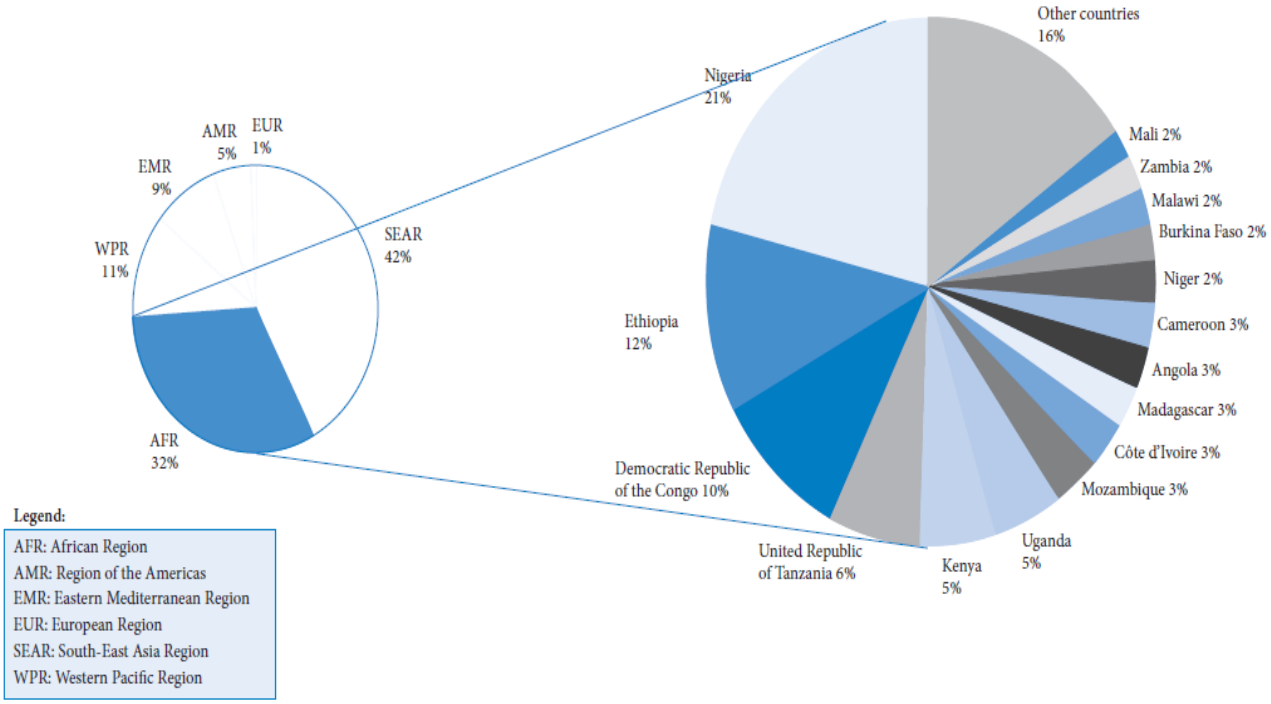

Figure 4. Proportion of children requiring preventive chemotherapy for STH in African region. Source. WHO STH report, 2001-2010

1.2.3 Malaria-Helminth coinfections

Climate, especially through the effects of temperature and rainfall, is said to be the major factor responsible for continental distribution of each of the major STH species.[17] Socio-economic factors are also important in the distribution of both malaria and STH, to the extent that over- dispersed distribution has been observed in malaria as well as STH infection in which only about 20% of children in a community receive 80% of new infections in a community.[18,19] Despite the high chances of co-infection between these 2 groups of parasites

in areas with overlapping distributions,[20,21] there are limited studies conducted so far to explore the possible outcome of their association. Earlier studies regarding their association reported that malnourished children infected with Ascaris were free of malaria and that deworming lead to increase in malaria attacks.[22,23] These findings were contradicted by later studies which suggested that helminth infected individuals were more prone to clinical malaria than helminth free subjects.[24,25] But Shapiroa *et al* [26]did not observe any association between malaria and helminth confection in a study carried out in Uganda

Clinical evidence of the additive nature of malaria and helminth induced anemia were demonstrated by studies among East African school children [27]as well as in Nigerian pregnant women.[28.] Paradoxically, hookworm which is the most important anemic causing helminth been postulated to protect against malaria.[29] It is therefore important to carry out more studies to get a deeper scientific insight into this subject matter. If it is conclusively resolved that there is a negative association, then mass deworming programs will need to be properly integrated with appropriate malaria treatment policies so as to avoid unwanted effect of treating either of the two in co-endemic region.

1.2.4 Opportunity for integrated malaria –helminth control strategies

On the basis of available evidence on the effectiveness and safety of providing deworming tablets to young children, it is now recommended that in areas of high helminth prevalence, preventive chemotherapy should be given to children from the age of 12 months. [30,31] This had helped made the school based mass deworming program a well-established protocol in many developing countries[3].Evidence from the benefit of helminth control among preschool children in Zanzibar indicates that anthelmintic treatment significantly reduced the prevalence of anemia and stunting among pre-SAC who received mebendazole every 3 months for 1 year. [32]

In addition to the use of residual house spraying and long lasting insecticide-treated nets, a promising new alternative malaria control strategy is intermittent preventive treatment in infants (IPTi). [33] Whereby doses of sulfadoxine-pyrimethamine (SP) are delivered to infants, irrespective of infection status, at the time of routine vaccination during the first year of life. In terms of the impact of IPTi on anemia, a Tanzanian study found a 50% protective efficacy against severe anemia among infants. [34] while another study in Ghana was associated with a significantly higher mean packed cell volume at 12 months of age[35]. Additionally, studies in West Africa have shown that seasonal IPT could be an effective malaria prevention strategy among children younger than 5 years of age in areas of seasonal malaria transmission. [36,37] Thus, anthelmintic treatment could potentially be co-administered with IPT in areas of seasonal malaria transmission. School-based treatment delivery programs offer several cost advantages because of the use of the existing school infrastructure and the fact that the children are accessible through schools.[38] This will also provide good opportunities for health promotion activities and teaching within schools to encourage good hygiene practices and awareness of malaria prevention methods, such as the use of sleeping under an insecticide treated net (ITN).

1.3 Diarrhea disease

As stated earlier, diarrhea is the 2nd cause of death among children globally, while in Africa and Nigeria, it ranked 3rd and 5th respectively. According to a research published by Prüss *et al* that estimated the burden of 107 major diseases and 10 risk factors at global and regional levels, "water, sanitation, and hygiene (WSH)" was considered as a single risk factor in poor countries constituting transmission partway for a group of diseases (Figure 5) including diarrhea, STH and malaria. [39]

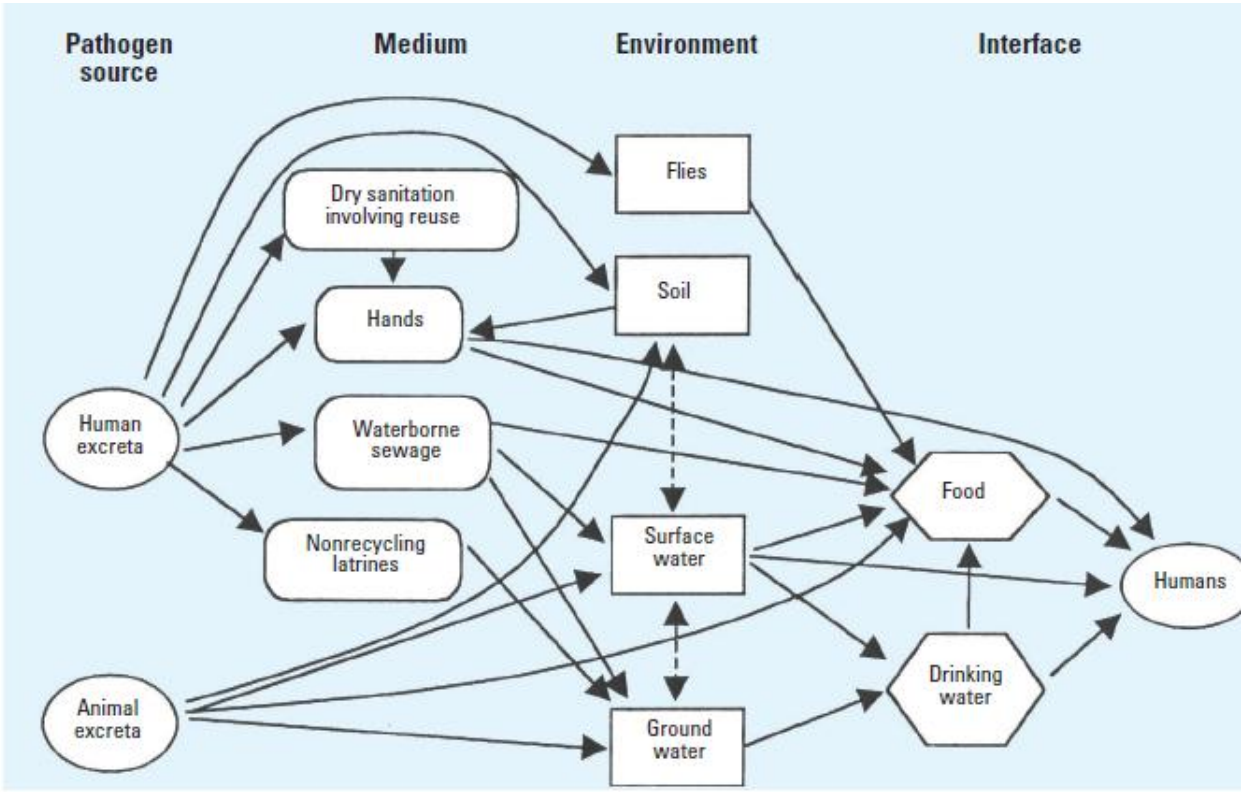

Figure 5. Transmission pathways of fecal-oral disease. Source. Prüss *et al*, 2002

Despite the success achieved in the drive towards the Millennium development Goals (MDGs), about 783 million people still do not use an improved drinking water source, while 2.5 billion do not use an improved sanitation facility, mostly in the poorest households and rural areas. [40] About 90 per cent of people who practice open defecation, the riskiest sanitation practice, live in rural areas. Nearly 90 per cent of deaths due to diarrhea worldwide have been attributed to unsafe water, inadequate sanitation and poor hygiene. The relatively unchanged incidence of diarrhea over the last 30 years despite the innovations in treatment and control suggest that knowledge gap exist about the burden of diarrhea causing pathogens.

The need for new research and specific treatment was recently highlighted by Gupta[41] who expressed fear regarding the recent stagnation in the coverage of ORS in many developing countries. He concluded that cost-effective and specific life-saving child survival interventions needed to be scaled up or recent gains in life expectancy may be threatened by

reversal. According to the 2005 World health report, despite the reduction in global child mortality rate from 146 per 1,000 in 1970 to 79 per 1,000 in 2003, the situation in African region remain worrisome because it showed the slowest rate of decline compared to other regions of the world [42]. For instance in 1980, the under 5 mortality rate in Africa was 4.3 times higher than the European region, but it was 7 times higher in 2003. Global estimate of diarrhea deaths also reduced from 4.6million in the 1980s[43] to 2.5 million in 2000.[44] Regrettably, morbidity rate did not show a parallel decline to the mortality rate. The global incidence in 2000 was 3.2 episodes per child, similar to what was obtainable in 1982[43] and 1992 [45] reviews. Diarrhea incidence was still similar to the more recent incidence in the African region as of the year 2010 which remain 3.3 episodes per child year [46]

In a recent case-control study to evaluate the burden and etiology of diarrheal disease in developing countries, one or more putative diarrhea pathogens were identified in 83% of the children with moderate-to-severe diarrhea and 72% controls respectively. Although a wide range of putative diarrhea pathogens were detected, only 4 contributed significantly to moderate or severe diarrhea cases. The four were rotavirus, *Cryptosporidium, Shigella,* and heat-stable enterotoxigenic *E coli* (ST-ETEC) in that order. Other parasites discovered from the study include *E. histolytica* and *Giardia*. Surprisingly *Giardia* was not significantly positively associated with diarrhea because it was identified more frequently in controls than in patients with moderate-to-severe diarrhea. [47]

Because of the high prevalence of Giardia and Cryptosporidium as intestinal pathogens, they were included in the World Health Organization's list of Neglected Disease Initiative.[48] A very important aspect of Giardia infection is the wide spectrum of clinical feature which can range from acute or chronic diarrhea to asymptomatic carriage.

For instance, studies in Egypt [49] and Mexico [50] revealed that stool carriage of Giardia were not associated with diarrhea. Another study in Israel showed that though Giardia carriage is not associated with diarrhea, its detection was significantly associated with growth impairment[51].

Apart from diarrhea, it can also present with nausea, weight loss, bloating and abdominal pain[52]. A carriage rate of 13.8 % was seen among Ethiopian healthy children in a recent study, [53] while a much lower prevalence of 1.9% was fond in diarrhea patients in another study in Saudi Arabia.[54] The wide variation in the carriage rate of Giardia among healthy and diarrheic children justifies the need for further investigations regarding the epidemiology of this pathogen. The finding by Fraser in the Israeli population also suggested that studies are really needed in Africa, where the problems of water and sanitation are more challenging, so as to evaluate the potential impact of such subclinical infections.

1.3.1 Diarrhea management and possible integration with malaria and STH treatment:- Integrated management of childhood illness (IMCI)

IMCI is an integrated strategy that is meant to promote accurate identification and combine treatment of major childhood illnesses in a child that presents at health care center in developing countries[55.] Because in most cases, the laboratory resources to identify individual infectious disease are not available, IMCI recommend clinical evaluation and combination of treatment for pneumonia, diarrhoea and malaria for children in rural settings. Impact assessment of IMCI strategy in many countries showed that it was more effective and also cheaper than the usual individualized approach. The IMCI recommended inclusion of ´´appropriate`` antimicrobial agent as part of the treatment for a child presenting with bloody diarrhea, severe diarrhea and/or fever.[55] The appropriate antibiotics should be selected based on the knowledge of probable causative agents in the local community. This again emphasizes the need to update the epidemiological data of potential diarrhea agents in developing countries with modern diagnostic tools such as real time-PCR which could potentially help IMCI in rational use of antimicrobial agents. [55]

1.4 Objective

The aim of the research is to study the epidemiologic aspect and effectiveness of school based mass deworming with albendazole using school teachers without formal training in Nigeria. (Publication III. In preparation)

1.4.1 Secondary objectives

1. Find out possible association between intestinal helminths and malaria among the school children (Publication 1)
2. Determine the molecular epidemiology selected protozoan pathogen in the study population (Publication 1I).

2.0 Results and Discussion

2.1 Paper I

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Epidemiological Study of the Association Between Malaria and Helminth Infections in Nigeria

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Abstract. The relationship between intestinal helminth infection and susceptibility to malaria remains unclear. We studied the relationship between these infections. Seven schools in Ilero, Nigeria referred all pupils with febrile illness to our study center for free malaria treatment during a 3-month study period. At the end, all pupils submitted a stool sample for microscopic investigation for helminth eggs. We used an unmatched case-control design to calculate the odds ratios for helminth infection in children with at least one attack of malaria (cases) and children with no malaria episodes during the study (controls). We recorded 115 malaria cases in 82 of 354 (23.2%), 16 of 736 (2.2%), and 17 of 348 (4.7%) children ages ≤ 5, 6–10, and 11–15 years old, respectively ($P = 0.001$). Helminth infection rate in cases was 21 of 115 (18.3%) compared with 456 of 1,327 (34.4%) in controls. Weighted odds ratio stratified by age group for helminth infection in cases versus controls was 0.50 (95% confidence interval = 0.2–0.84, $P < 0.01$). *Ascaris* and hookworm were the most common helminths detected, with prevalence rates of 14 (12.2%) and 6 (5.2%) among cases compared with 333 (25.1%) and 132 (10.0%) in controls, respectively ($P = 0.001$). The negative association between helminth infection and malaria may be of importance in the design of deworming programs.

INTRODUCTION

About one-half of the world's population is at risk of contracting malaria, with an annual mortality rate up to 0.5 million.[1] Malaria remains one of the most common killers of young children in sub-Saharan Africa, where 90% of malaria-related deaths occur.[1] Although the World Health Organization (WHO) reports that malaria deaths have been reduced by 33% in the African region, a child still dies every 1 minute as a result of the disease.[2] However, up to one-third of the world population (more than 2 billion people) are infected with intestinal parasites, and about 300 million people are severely ill; at least 50% are school-aged children.[3] Although intestinal helminths are only rarely a direct cause of death, their public health impact is substantial because of the number of people affected.[4] About 4.98 million disability-adjusted life years (DALYs) are attributed to intestinal helminths, and these infections, thus, represent a substantial economic burden.[5] The most common intestinal helminths infecting humans include *Ascaris*, hookworm, and *Trichuris*; all three are widely distributed in tropical countries, infecting 1.4, 1.3, and 1.0 billion people, respectively.[6]

Malaria and intestinal helminths overlap extensively in their epidemiological distributions, and coinfections are frequently seen.[7,8] The possible interactions between coinfecting intestinal helminths and plasmodia have been investigated over the decades with conflicting results. According to a review that summarizes the outcomes of malaria–helminth coinfection, 13 studies suggested that intestinal helminths were associated with protection from malaria, whereas 8 studies showed increased malaria severity and incidence in coinfected individuals. Finally, five studies found no association between malaria and helminths infestation.[9]

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This study was designed to identify the association of intestinal helminths with malaria incidence in a semirural area of Nigeria with a goal of contributing to the body of evidence on the role played by intestinal helminths in malaria epidemiology.

MATERIALS AND METHODS

Study site. The study was carried out in the town of Ilero (latitude: 8°4′0 N; longitude: 3°21′0 E) in Oyo State, southwestern Nigeria.[10] The vegetation of Ilero is Guinea Savanna, with a distinct rainy season from April to September and a dry season from October to March. Farming is the predominant occupation of the inhabitants of this community. The town has a population of about 35,000 inhabitants.[11]

Study population. All school- and pre–school-aged children from seven public primary schools in Ilero were recruited for the study. The schools and parents in the community were asked to refer children with febrile illness to our study center for free malaria treatment during a 3-month study period in the malaria transmission season (May to July of 2013). At the end of the study, the schools were visited to screen the children for intestinal parasites. Because all of the schools also have nursery facilities, it provided us with an opportunity to enroll children below the age of 5 years. Although as indicated below, participation in the study was strictly voluntary, all parents consented to their children's participation, likely because the access to free and timely treatment was considered a common good. Thus, no selection of study participants was done.

Study design. The study was designed as a case-control study involving children with and without malaria (cases and controls, respectively) over a 3-month period. Inclusion criteria were all of the children at all seven schools who submitted a stool sample at the end of the study period. Cases were febrile children referred to our study center with laboratory-confirmed malaria, whereas controls were the children who had no malaria. Controls were further questioned at the end of the third month to ensure that they had not experienced episodes of febrile illness during this period. Children with an undiagnosed febrile illness during the study period that did

not test positive for malaria but received empiric antimalarial therapy were excluded.

Diagnosis of intestinal helminth infestations. At the end of the project, children in the case and control arms of the study were asked to submit stool samples, which were immediately preserved with 5% formalin, later processed, and examined by microscopy of direct smears of iodine-stained preparations for ova and parasites. As quality control, all samples were examined by two independent microscopists (a laboratory physician and a laboratory technologist); any discrepancies were resolved by microscopy of formol-ether–concentrated specimens by a third laboratory technologist.

Malaria diagnosis. Clinically suspected cases of malaria were subjected to laboratory tests. A rapid malaria diagnostic kit (SD Bioline Malaria *Plasmodium falciparum*; Standard Diagnostics, Gyeonggi-do, Korea) was used to screen clinically positive cases, which were later confirmed by light microscopy of blood smears.

Ethical issues. Ethical approval for the study was issued by the research and ethical committee of the Federal Teaching Hospital, Abakaliki, Nigeria. Written informed consent was also obtained from all of the parents or guardians of the participating pupils.

Statistical analysis. Data were entered into EPI-info (3.5.3) statistical software. Descriptive statistics were used to cross-tabulate the variables, and we used an unmatched case-control design to calculate the odds ratio (OR) for helminth infection in malaria patients (cases) and children with no malaria episodes during the study (controls). Because malaria prevalence was highest in younger age groups and helminth infestations were most common in older age groups, the data analysis was stratified by age group to avoid confounding caused by age difference. A P value < 0.05 in the χ^2 test with Yate's correction was considered significant. The study had approximately 90% power at 95% confidence to show a difference in helminth prevalence from 33% in controls to 20% in cases assuming a 10:1 ratio between controls and cases.

RESULTS

Cases and controls were enrolled from the total unselected population of the seven schools tested for intestinal helminths; 25 pupils who were not present for stool collection because of travel and 5–10 children per school who could not produce a stool sample were excluded. None of these children were among the malaria cases. Finally, five children with a febrile illness that could not be diagnosed were excluded. After exclusion, the total number of children who participated in the study was 1,442; 126 suspected malaria cases were tested by rapid diagnostic test (RDT) and microscopy during the 3-month study period, of whom 115 (91.3%) children had the malaria diagnosis confirmed. Two children presented with a second malaria episode toward the end of the third month, but they were not counted, because cases were defined as having at least one episode of malaria. All of the suspected malaria cases were tested by RDT as well as microscopy, and there was 100% agreement between the two laboratory methods. Malaria incidence was significantly different between age groups, with a relative risk of malaria in children under 5 years old compared with older children of 7.6 (95% confidence interval [95% CI] = 5.2–11.2, $P < 0.001$).

The prevalence of intestinal helminths was 476 of 1,442 (33.0%) children. *Ascaris* was the most common helminth identified in this study (22.3%) followed by hookworm (7.8%). Other species were rare, and 23 (1.6%) of the children were coinfected with two helminth species (Table 1).

The distribution of helminth species was similar in cases and controls (Table 2), but the prevalence of helminth infection was lower in cases (21 of 115; 18.3%) than among controls (455 of 1,327; 34.3%).

We noted that the prevalence of intestinal helminth infection differed significantly between age groups. For instance, the prevalence of helminths in control children 5 years old or younger was 29.8%, the prevalence of helminths in control children 6–10 years old was 37.6%, and the prevalence of helminths in control children 11–15 years old was 30.5% (Table 3) ($P = 0.01$). To compensate for confounding caused by different age distributions of malaria and helminth infection, we stratified our analysis by age. In children < 5 and 6–10 years old, the prevalence of helminth infection was significantly higher in controls than cases ($P = 0.03$ and $P = 0.005$, respectively), whereas no difference could be found in older children ($P = 0.5$) (Table 3). The weighted OR for helminth infection in cases

TABLE 1
Distribution of malaria cases and intestinal helminth species across age groups

	Age group (years), *N* (%)				
	≤ 5	6-10	11–15	> 15	Total
Malaria positive	82 (23.2)	16 (2.2)	17 (4.9)	0 (0)	115 (8.0)
Malaria negative	272 (76.8)	718 (97.8)	333 (95.1)	4 (100)	1,327 (92.0)
Total	354 (100)	734 (100)	350 (100)	4 (100)	1,442 (100)
Helminths					
Ascaris sp.	74 (20.1)	184 (25.1)	61 (17.4)	2 (50.0)	321 (22.3)
Hookworm	15 (4.2)	58 (7.9)	39 (11.1)	0 (0)	112 (7.8)
T. trichuria	0 (0)	5 (0.7)	2 (0.6)	0 (0)	7 (0.5)
Enterobius vermicularis	0 (0)	5 (0.7)	0 (0)	0 (0)	5 (0.4)
Ascaris sp. and hookworm	2 (0.6)	15 (2.0)	6 (1.7)	0 (0)	23 (1.6)
Ascaris sp. and *T. trichiura*	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (0.1)
Ascaris sp. and *E. vermicularis*	0 (0)	2 (0.3)	0 (0)	0 (0)	2 (0.1
Taenia sp.	3 (0.9)	0 (0)	0 (0)	0 (0)	3 (0.21)
Hookworm and *E. vermicularis*	0 (0)	1 (0.1)	0 (0)	0 (0)	1 (0.1)
Hookworm and *T. trichiura*	0 (0)	0 (0)	1 (0.3)	0 (0)	1 (0.1)
Helminth positive	95 (26.8)	270 (36.8)	109 (31.1)	0 (0)	476 (33.0)
Helminth negative	259 (73.2)	464 (63.2)	241 (68.9)	2 (100)	966 (67.0)
Total	354 (100)	734 (100)	350 (100)	4 (100)	1,442 (100)

TABLE 2
Distribution of intestinal helminth species among cases and controls

Malaria status	Intestinal helminths, *N* (%)												
	Asc	Hw	Tt	Ev	A+H	A+T	A+E	Tae	H+E	H+T	H.pos	H.neg	Total
Case	12 (10.4)	4 (3.5)	1 (0.9)	0 (0)	2 (1.7)	0 (0)	0 (0)	2 (1.7)	0 (0)	0 (0)	21 (18.3)	94 (81.7)	115 (100)
Control	309 (23.3)	108 (8.1)	6 (0.5)	5 (0.4)	21 (1.6)	1 (0.1)	2 (0.2)	1 (0.1)	1 (0.1)	1 (0.1)	455 (34.3)	872 (65.7)	132 (100)
Total	321 (22.3)	122 (8.5)	7 (0.5)	5 (0.4)	23 (1.6)	1 (0.1)	2 (0.1)	3 (0.2)	1 (0.1)	1 (0.1)	476 (33.0)	966 (67.0)	1,442 (100)

A+E = *Ascaris* sp. and *E. vermicularis*; A+H = *Ascaris* sp. and hookworm; Asc = *Ascaris* sp.; A+T = *Ascaris* sp. and *T. trichiura*; Ev = *Enterobius vermicularis*; H+E = hookworm and *E. vermicularis*; H.neg = helminth negative; H.pos = helminth positive; H+T = hookworm and *T. trichiura*; Hw = hookworm; Tae = *Taenia* sp.; Tt = *T. trichuria*.

versus controls across all age groups was 0.50 (95% CI = 0.2–0.84; stratified χ^2 test $P < 0.01$).

Females tended to have higher incidence of malaria (68 of 724; 9.4%) than males (47 of 718; 6.5%), with a relative risk of malaria in females compared with males of 1.43 (1.00–2.05; $P = 0.06$).

DISCUSSION

Our study supports the possible protective effect of helminth infections against malaria. Most (71.3%) of the children who experienced a clinical malaria attack during the observation period were in the age group ≤ 5 years old, which is in agreement with the usual trend in malaria epidemiology.[2,12] This age group is most susceptible to malaria because of the time that it takes to acquire immunity to variant surface antigens of *P. falciparum*.[13] Notably, the protective effect of helminth infections was present in both the youngest age group, where the risk of malaria was high, and the 5- to 10-year-old age group, where the risk was low. Only the children older than 10 years old did not seem to benefit from a protective effect of helminth infection against malaria. Our findings contrast an intervention study from Madagascar, where bimonthly deworming resulted in a significant increase in *P. falciparum* density in cases compared with controls.[14] This change was seen only in 5- to 14-year-old children, and there was no effect of treatment in children ages 6 months to 4 years old or subjects > 15 years of age.[14]

In our study, males had a lower incidence of malaria than females (40.9% and 59.1%, respectively). This difference was borderline significant. This is not in agreement with a study in Uganda,[15] which showed that confirmed malaria was lower among girls (52.3%) than boys (57.3%), and it had no bearing on the effect of helminth coinfection (data not shown).

There are wide variations in the prevalence of and helminth species involved in coinfections with malaria across study sites. Thus, the helminth coinfection rate among the malaria cases in our study was lower than what was observed in a recent study in Tanzania, where a 60% coinfection rate was seen among schoolchildren. In this study, a similar protective effect of malaria coinfection with *Schistosoma* spp. in particular was noted, whereas there was an opposite trend for hookworms.[16] Despite methodological differences between studies, geographic differences indicate a need to test helminth–malaria interactions in a range of settings. This study was, thus, undertaken as part of an evaluation of the policy to routinely deworm schoolchildren in Nigeria.

Our findings strengthen the worry that regular deworming of children in endemic areas could potentially be putting the children at increased risk of malaria.[17] It is particularly worrying that the protective effect of helminth coinfection was seen in the youngest children, who are at the highest risk of malaria. Because deworming is now a routine public health intervention in Nigeria,[18] it is unlikely that permission would be granted to conduct a randomized trial of the effect of deworming on malaria risk. Furthermore, historical controls cannot be used to compare malaria risk before and after deworming in a treatment series, because malaria transmission changes with season and annual variation. However, the study raises the question of whether deworming should routinely be combined with intermittent preventive treatment of malaria in children (IPTc)[19] or other interventions aimed at reducing malaria risk. As for many other aspects of public health interventions in Africa, this calls for an integrated approach to health problems instead of the common vertical campaigns.[20] In addition, additional studies should scrutinize the effect on malaria risk and other health consequences of deworming in groups with or without simultaneous administration of IPTc.

The results of our study also reinforce the fact that the helminth status of a potential malaria vaccine target population must be critically evaluated with deeper insight into the outcomes of helminth–malaria interactions before successful design and deployment of effective vaccines against malaria are undertaken. Helminths have been shown to reduce the efficacy of a number of vaccines, such as for bacille calmette-guérin (BCG),[21] tetanus,[22] and cholera.[23] The immunogenicity of human immunodeficiency virus (HIV)-1 and malaria candidate vaccines[24–26] was also shown to be reduced by helminth coinfection. Inhibition of vaccine-elicited immune responses is generally attributed to T-helper lymphocyte (Th)2 polarization and interleukin-4 (IL-4) production associated with most helminth infections.[26] Several studies have shown that

TABLE 3
Prevalence of helminth infestations in cases and controls by age group

Age group (years)/malaria status	*N* (%)	Helminth status, *N* (%)	
		Positive	Negative
< 5			
Positive	82 (23.2)	14 (17.1)	68 (82.9)
Negative	272 (76.3)	81 (29.8)	191 (70.2)
6–10			
Positive	16 (2.2)	0 (0)	16 (100)
Negative	718 (97.8)	270 (37.6)	448 (62.4)
11–15			
Positive	17 (4.9)	7 (41.2)	10 (58.8)
Negative	333 (95.1)	102 (30.5)	232 (69.5)
> 15			
Positive	0 (0)	0 (0)	0 (0)
Negative	4 (100.0)	2 (50.0)	2 (50.0)

elimination of helminth parasites before immunization seems to restore normal vaccine responsiveness.[26–29]

In conclusion, our study adds additional support to earlier observations of an association between the presence of intestinal helminth infection and the development of clinical malaria.

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2.2 Paper II

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Molecular Detection of the Carriage Rate of Four Intestinal Protozoa with Real-Time Polymerase Chain Reaction: Possible Overdiagnosis of *Entamoeba histolytica* in Nigeria

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Abstract. Diarrhea remains the second largest killer of children worldwide, and Nigeria ranks number two on the list of global deaths attributable to diarrhea. Meanwhile, prevalence studies on potentially diarrheagenic protozoa in asymptomatic carriers using molecular detection methods remain scarce in sub-Saharan countries. To overcome sensitivity issues related to microscopic detection and identification of cysts in stool concentrates, real-time polymerase chain reaction (PCR) was used to analyze genomic DNAs extracted from stool samples from 199 healthy school children for *Entamoeba histolytica*, *E. dispar*, *Giardia intestinalis*, and *Cryptosporidium*. Questionnaires were administered for epidemiological data collection. *E. histolytica* was not detected in any of the samples, whereas *Giardia* (37.2%), *E. dispar* (18.6%), and *Cryptosporidium* (1%) were found. Most of the children sourced their drinking water from community wells (91%), while the majority disposed of feces in the bush (81.9%). Our study is the first to use real-time PCR to evaluate the epidemiology of *E. histolytica*, *Giardia*, and *Cryptosporidium* in Nigeria where previous studies using traditional diagnostic techniques have suggested higher and lower carriage rates of *E. histolytica* and *Giardia*, respectively. It is also the first study to accurately identify the prevalence of common potentially diarrheagenic protozoa in asymptomatic carriers in sub-Saharan Africa.

INTRODUCTION

Diarrhea is the second leading cause of death among children aged less than 5 years, globally causing about 1.5 million deaths each year. The disease kills more young children than acquired immunodeficiency syndrome (AIDS), malaria, and measles combined.[1] Africa and south Asia bear more than 80% of the global share of child deaths because of diarrhea, while country-wise, Nigeria with 151,700 diarrhea-related fatalities per year, is currently ranked number two, topped only by India.[2] Apart from direct mortality, persistent diarrhea has been associated with malnutrition, poor growth, as well as systemic infections of the respiratory and urinary tracts.[3,4] Although intestinal protozoal infections because of *Entamoeba histolytica* and *Giardia* are among the wide range of pathogens responsible for diarrhea, rotavirus, *Cryptosporidium*, and bacterial agents such as *Escherichia coli* and *Shigella* are the most common etiologic agents.[5] A recent survey of data on childhood deaths collected between 2000 and 2012 in seven low- and middle-income countries showed that persistent diarrhea requiring investigation accounted for more than 30% of diarrheal deaths in infants aged 1–11 months at 5/7 sites and more than 25% of deaths in children aged 1–4 years at 3/5 sites for which data were available. It was further revealed that at six of the seven study sites, 70–100% of children with persistent diarrhea who died had been seen in a health-care facility, while more than 50% of them had received oral rehydration solutions or intravenous fluids.[6]

Protozoa are important causes of persistent diarrhea,[2] and current emphasis on rehydration fluid is adequate only for acute diarrhea agents such as rotavirus, which is self-limiting if the lost fluid and electrolytes are adequately replaced. These observations raise fundamental questions as to the quality of diagnosis and care provided to these children.

Apart from overall issues with timely and accurate diagnosis of intestinal protozoal infections in sub-Saharan Africa, the lack of ability to differentiate between *E. histolytica* and nonpathogenic amoebic species has erroneously inflated the etiologic role of the former as a diarrhea-causing agent,[7] potentially leading to mismanagement of diarrheal disease, while the real causative agents may continue to spread undeterred,[8] with the additional risk of emergence of organisms that are antimicrobially resistant.[9] Organism-specific stool testing is also important for public health reasons because results can serve as early warning of an impending outbreak.[10]

The diagnosis of *E. histolytica* infection has traditionally relied on microscopic examination of fresh or fixed stool specimens.[11] Meanwhile, microscopy has at least two major limitations; one is its suboptimal sensitivity, which is about 60%,[12] while the second is its inability to distinguish potentially pathogenic *E. histolytica* from morphologically identical but nonpathogenic *E. dispar*, *E. moshkovskii*, and other quadrinucleate cysts of *Entamoeba*.

Serological methods have become useful for detection of *E. histolytica* infections in developed countries. However, serological tests are unable to definitively distinguish past from current infections in developing countries where amoebiasis is more common. Some of the serological tests are either costly, time consuming, require special skills, insensitive, or unspecific, or suffer from a combination of these setbacks.[13]

Newer approaches, including polymerase chain reaction (PCR) and antigen-based enzyme-linked immunosorbent assays (ELISAs), have emerged as means of solving the challenge of detecting *E. histolytica*.[12]

Several studies have been carried out to compare the performance of the newer methods, showing that PCR has better sensitivity and specificity in the detection of *E. histolytica*.[12,14–16]

Strongly endorsed by the World Health Organization (WHO),[17] the use of PCR is currently the method of choice

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for clinical and epidemiological studies of amebiasis in developed countries. However, the problem of overdiagnosis of *E. histolytica* remains a major problem in developing countries despite the fact that species-specific PCR methods have been available for more than a decade. The use of laboratory methods not enabling differentiation between *E. histolytica* and nonpathogenic amoebae has largely been responsible for uncertainty regarding the epidemiology of amebiasis as well as the frequently quoted global prevalence rate of this pathogen.[15]

The epidemiology of *E. histolytica* infections in Nigeria hence remains unknown because nonmolecular methods are still being used in investigations, with prevalence figures ranging from 35.4% to 72%,[18,19] typically around 40% as estimated by one of the earliest studies some 25 years ago.[20]

There are very few studies on the prevalence of intestinal protozoa in asymptomatic carriers. Such studies are critical to gaining an understanding of the clinical and public health significance of intestinal protozoa. To the best of our knowledge, this study is the first attempt to establish the epidemiology of the prevalence of not only *E. histolytica*, but also *E. dispar*, *Giardia*, and *Cryptosporidium* in healthy children in Nigeria using real-time PCR.

MATERIALS AND METHODS

Study site and study population. Sample collection was carried out in the town of Ilero (latitude, 8°4′ 0N; longitude, 3°21′ 0E), a semirural community in southwestern Nigeria. The vegetation of Ilero is Guinea savanna with a distinct rainy season from April to September and a dry season from October to March. Farming is the predominant occupation of the inhabitants of this community. The town has a population of about 35,000 inhabitants.[21] All the schools also run preschool nursery facilities, thereby giving us the opportunity to sample children below the age of 6 years as well. There were seven primary schools in this community where stool samples were collected during a mass deworming program. About 1,442 pupils submitted stool samples from which 199 were randomly selected for DNA extraction and PCR-based screening for selected intestinal protozoa.

Questionnaires. Questionnaires were administered to children and their parents/guardians to obtain information about sociodemographic data such as age, religion, ethnic group, father's occupation, source of drinking water, means of feces disposal, and so on. The information obtained was entered into an Excel spreadsheet for future analysis.

DNA extraction. The QIAamp DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany) was used to extract DNA from 200 mg frozen stool samples according to the manufacturer's instructions but with some modifications. The modification carried out involved the addition of about 0.3 g zirconium-silica beads (diameter, 0.1 mm; Biospec Product Inc., Bartlesville, OK) to each tube just before the first heating step at 95°C for 5 minutes, after which the sample was shaken at 30 Hz for 6 minutes by a TissueLyser (QiagenRetsch GmbH, Hanover, Germany). This modification was carried out to increase DNA yield for downstream processing.[22]

Real-time PCR. Real-time PCR for *Giardia*, *E. histolytica*, and *E. dispar* was performed using the primers and protocols described by Verweij and others.[23,24] For *Cryptosporidium* real-time PCR, an in-house protocol (Stensvold, unpublished) was used for the amplification of the small subunit (SSU) rRNA gene of the genus *Cryptosporidium*.

Positive and negative controls were included in each real-time PCR, run as part of the standard protocol of the Laboratory of Parasitology, Statens Serum Institut, Copenhagen, Denmark. Furthermore, the real-time assays had previously been validated by spiking assays (sensitivity) and confirmatory PCR and sequencing of positive samples (specificity).

To corroborate our real-time PCR findings, 50 of the samples were also tested using an alternative method. The TechLab *E. histolytica* II kit (Techlab, Blacksburg, VA)—a second generation monoclonal ELISA-based test for detecting *E. histolytica* galactose adhesin in fecal samples—was used for *E. histolytica*. The test was performed according to the manufacturer's instructions. ELISA results were analyzed by an automatic microtiter plate reader (Anthosht III; AnthosLabtec, Germany) at 450 nm. Positive results were defined as an optical density reading of ≥ 0.05 after subtraction of the negative control optical density.

For both *Cryptosporidium* and *Giardia*, a direct immunofluorescence (DIF) microscopy test, Crypto/*Giardia* Cel, (Cellabs Pty Ltd., Brookvale, New South Wales, Australia) was used. The tests were carried out in line with the manufacturer's instructions, incorporating positive and negative controls into each analysis.

Ethical issues. Ethical approval for the study was issued by the research and ethical committee of the Federal Teaching Hospital, Abakaliki, Nigeria. Written informed consent was also obtained from all the parents or guardians of the participating pupils. Parents and guardians were told that participation in our study was voluntary and all participating children were given free deworming tablets irrespective of stool sample submission.

Statistical analysis. Data were entered into the EPI-info (3.5.3) statistical software. Descriptive statistics were used to cross-tabulate the variables. The χ^2 test with Yate's correction was used for comparison of categorical data and Student's *t* test was used for comparison of normally distributed data. A P value < 0.05 was considered statistically significant.

RESULTS

Most of our study subjects were aged 6–10 years (61.3%) followed by those who were 5 years or less (Table 1). The majority of the children (90.5%) were from the Yoruba ethnic group.

Of the study subjects, 114 (57.3%) were from Christian homes, and 55.3% were males.

Analysis of the data pertaining to the occupation of the fathers of these children showed that farming (51.2%) was the most common occupation in this community followed by trading (17.6%). Community wells (91%) represented the most common source of drinking water followed by water sealed in nylon sachets (7.0%). Tap water was not available in the community. The major form of sewage disposal was in the bush (81.4%) followed by the use of pit latrines (15.1%).

No DNA of *E. histolytica* was detected in any of the stool samples, while *E. dispar* was identified in the stool of 37 (18.6%) children by real-time PCR. *Giardia*, detected in 74 (37.2%) of the children, was the most common intestinal protozoon identified, while only two (1.0%) cases of *Cryptosporidium* were observed (Table 1).

Giardia (41.3%) was more prevalent in children below the age of 6 years whereas *E. dispar* was more common in the

TABLE 1
Distribution of intestinal protozoa in relation to sociodemographic characters of the study population

Characteristic	*Entamoeba histolytica* N (%)	*E. dispar* N (%)	*Giardia* N (%)	*Cryptosporidium* N (%)
Age (years)				
< 6, N = 46	0	3 (6.5)	19 (41.3)	0
6–10, N = 122	0	27 (22.1)	43 (35.6)	2 (1.6)
11–14, N = 31	0	7 (22.5)	12 (38.7)	0
Sex				
Male, N = 110	0	19 (17.3)	40 (36.4)	1 (0.9)
Female, N = 89	0	18 (20.2)	34 (38.2)	1 (1.1)
Religion				
Christianity, N = 114	0	22 (19.3)	40 (35.1)	1 (0.9)
Islam, N = 85	0	15 (17.5)	34 (40.0)	1 (1.1)
Tribe				
Yoruba, N = 180	0	33 (18.3)	66 (36.7)	2 (1.1)
Hausa, N = 6	0	0	0	0
Ibo, N = 10	0	3 (30.0)	7 (70.0)	0
Fulani, N = 3	0	1 (33.3)	1 (33.3)	0
Occupation				
Farming, N = 102	0	19 (18.7)	38 (37.3)	2 (2.0)
Trading, N = 35	0	7 (20.0)	18 (51.4)	0
Artisan, N = 29	0	8 (27.6)	9 (31.0)	0
Others, N = 28	0	3 (20.0)	9 (32.1)	0
Nil, N = 5	0	0	0	0
Toilet facility				
Pit latrine, N = 30	0	4 (13.3)	11 (36.7)	1 (3.3)
Water closet, N = 6	0	1 (16.7)	1 (16.7)	0
Bush, N = 162	0	32 (19.8)	62 (38.0)	1 (0.6)
Drinking water				
Well, N = 181	0	33 (18.2)	68 (37.6)	2 (1.1)
Nylon sachet water N = 14	0	3 (21.4)	4 (28.6)	0
Stream N = 4	0	1 (25.0)	2 (50.0)	0

older age groups. The two cases of *Cryptosporidium* occurred in children aged 6–10 years. *Giardia* was more common in females (38.2%) and children from the Ibo (70.0%) ethnic group. The two *Cryptosporidium* cases were seen in children from farming homes, while *Giardia* was most common in children whose fathers were traders (51.4%). *E. dispar* (19.8%) and *Giardia* (38.0%) were more common in children who disposed feces in the bush, while these two protozoa were also more prevalent in children who sourced their drinking water from the stream, (25% and 50%, respectively; Table 1). However, no statistically significant differences between any of the groups were found ($P > 0.05$).

Supplementary laboratory tests were performed on 50 selected samples (age groups < 6 years, $N = 10$; 6–10 years, $N = 30$; 11–14 years, $N = 10$). Of these 50 samples, two were PCR-positive for *Cryptosporidium*, 24 were PCR-positive for *Giardia*, and 30 were PCR-positive for *E. dispar*. All 50 samples were negative for *Cryptosporidium* by DIF microscopy, including the two samples that were previously positive by real-time PCR. All the samples that were real-time PCR-negative for *Giardia* were also negative by DIF microscopy, while 10/24 samples positive for *Giardia* by real-time PCR were positive by DIF. Hence, 14/24 real-time PCR-positive samples were negative by DIF microscopy for *Giardia*, yielding a DIF sensitivity of 41.67% compared with real-time PCR.

The mean cycle threshold (CT value) for the *Giardia* real-time PCR was 35.5 with a range of 28.6–44.0 and a standard deviation (SD) of 3.55, while the mean CT value for *E. dispar* was 35.5 with a range of 27.4–42.5 and an SD of 4.6.

The mean CT value for DIF-microscopy-positive samples was 31.5 (SD, 1.7), which was significantly lower than that for DIF-microscopy-negative samples (mean, 37.5 [SD, 2.5]; $P < 0.001$), suggesting that DIF-microscopy-positive samples had a higher parasite load compared with microscopy-negative samples.

Regarding *E. histolytica*, all 50 samples (comprising 30 *E. dispar* PCR-positive samples and 20 *E. dispar* PCR-negative samples) were negative by antigen ELISA, confirming PCR results and the low prevalence of this species in healthy children.

DISCUSSION

Our findings showed a surprisingly low prevalence of *E. histolytica* carriage in Nigerian children compared with previous studies. Although the absence of *E. histolytica* in our study population was initially puzzling given the fact that majority of these children source their drinking water from community wells (91%) and also dispose their feces in bushes (81.4%), the high prevalence of *Giardia* (37.2%) showed that the lifestyle in this community still involves a high degree of transmission of intestinal pathogens. Various types of intestinal helminthes[22] and a very high prevalence of *Blastocystis* (Efunshile and Stensvold, unpublished data) were also discovered in this community, which may indeed evince the mode of unhygienic fecal disposal and fecal–oral transmission. There was no significant association between the prevalence of giardiasis and any of the sociodemographic characters in our study population, but any such associations may be blurred by extensive overall risk behavior. The trend toward a higher prevalence of *Giardia* in younger children, suggesting a particular risk behavior in this group, was in agreement with observations from a Ugandan study.[25]

The low carriage rate of *E. histolytica* in our study is in contrast to previous studies in Nigeria where higher prevalence figures were reported.[18–20] These studies were all based on

microscopic stool investigations that do not discriminate between *E. dispar*, which was also prevalent in our study, and *E. histolytica*. In addition, one of the studies focused on patients with diarrhea.[18] Since we have used the most sensitive and specific diagnostic tool (PCR) recommended by the WHO, shown to be able to detect a single trophozoite of *E. histolytica* compared with diagnostic alternatives,[15] at least we can say that there is no sign of a reservoir of asymptomatic carriage among children as evidenced by this study.

Our result is in agreement with molecular studies in many other developing countries, for example Ethiopia, where Kebede and others used real-time PCR to investigate 214 stool samples that were reportedly positive by microscopy for *Entamoeba* only to find that none of the samples were positive for *E. histolytica* DNA.[26] Also, a molecular survey of stool samples from food handlers previously diagnosed with amebic infection by microscopy in Tunisia eventually showed that no one was infected by this species.[27] In another study in Brazil, a molecular reevaluation of 227 stool samples microscopically positive for the *E. histolytica*/*E. dispar* complex showed that only *E. dispar* was present,[28] and a recent study showed that *E. histolytica* was absent among a selected, apparently healthy, population of children in Ghana, contrary to previous belief.[29] The availability of molecular methods to distinguish between *E. dispar* and *E. histolytica* has thus thrown into question the commonly accepted figure of 500 million *E. histolytica* infections worldwide,[7] suggesting that the true global prevalence may have been largely overestimated.

It is important to correctly diagnose diarrheal patients not only to reduce the morbidity and mortality of amebiasis but also to minimize the undue treatment of patients infected with *E. dispar* and potentially other species of *Entamoeba* in general to better be able to match treatment with diarrhea-causing microorganisms. For instance, overdiagnosis of *E. histolytica* implies that colitis resulting from bacterial agents such as *Campylobacter* and *Shigella* species will probably be inappropriately treated with antiprotozoal drugs, while failure to specifically diagnose *E. histolytica* is associated with fatal consequences.[30,31]

There are still large gaps in the knowledge of species prevalence rates in different regions of the world, particularly in the African continent where very few studies are being performed using molecular methods. To address this deficit, there is a need to implement species-specific diagnosis of *E. histolytica*. Moreover, there is a need to strengthen collaboration between researchers from developing countries and those from developed countries so as to be able to effectively identify the true epidemiology of these parasites, and hopefully produce novel, accurate, sensitive, easy-to-use, inexpensive, sustainable, and highly applicable diagnostics that circumvents the requirement for advanced technological infrastructure.[7] This will in turn improve rational use of antimicrobial agents and treatment outcomes. The TechLab II ELISA could be useful as a diagnostic tool to rule out *E. histolytica* in an area where nonpathogenic amoeba species such as *E. dispar* are endemic. The agreement between this method and real-time PCR in our study population is similar to the observation of the research group of Roy[32] and that of Visser.[33] However, because of the lack of positive results our study could not be used to validate this method.

The low prevalence of *Cryptosporidium* (1%) noted in this study was not surprising since this particular protozoon has been shown to be more prevalent in immunocompromised individuals and in cases of diarrhea.[5] For instance, in Tanzania, Cegielski and others found a prevalence of 15% in human immunodeficiency virus (HIV)–infected children with diarrhea, while *Cryptosporidium* was not found among healthy controls.[34]

Similarly, among HIV-positive Ugandan children with diarrhea, a prevalence of 73.6% was reported compared with 5.9% among the controls. The Uganda study further showed that *Cryptosporidium* was essentially associated with low CD4 cell counts.[35] Elsewhere, cryptosporidiosis has been shown to be a marker of low CD4 count.[36] However, in contrast to our results, a high *Cryptosporidium* carriage rate was reported in community-based studies in Bolivia[37] (31.6%) and Korea[38] (3.3%) among healthy individuals. Over-crowding and direct contact with cattle was attributed to the high rate in their studies, but it should be noted that microscopy—not PCR—was used as the screening method in both studies.

The high prevalence of *Giardia* recorded in our study was similar to the findings of Kotloff and others in the recently published data from Global Enteric Multicenter Study (GEMS)[5] where *Giardia* was surprisingly discovered to be significantly more prevalent in the control group of children without diarrhea than in the cases. A systematic review by Abba and others discovered that the various types of intestinal pathogens commonly associated with persistent diarrhea in children can also be asymptomatically carried with a similar frequency in children without diarrhea and came up with the recommendation that new research will be needed across countries to help map the causes and be able to explore effective options for empirical treatment.[39] Previous studies based on traditional microscopy by Uneke and others (2.3%)[40] and Ukpai and others (1.7%)[41] in Nigeria and also by Wegayehu and others from Ethiopia (13.8%)[42] have shown a lower carriage rate of *Giardia*. This led us to compare the real-time PCR result with another highly specific test for *Giardia*, that is, DIF microscopy. Using this technique, the estimated carriage rate dropped to 15.5% in our study population. The observation of fewer *Giardia*-positive samples and no *Cryptosporiodium*-positive samples by DIF microscopy compared with real-time PCR was not unexpected; a previous study showed PCR to be the more sensitive method for detecting these organisms.[43]

The high CT values in DIF-microscopy-negative cases indicate that PCR testing for *Giardia* may be too sensitive to discriminate between clinically relevant infection and asymptomatic carriage. Further studies should address this question and also clarify the potential importance of an asymptomatic carrier state.

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2.3 Paper III (In preparation)

Effectiveness of mass deworming with albendazole by school teachers without formal training in Nigeria

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Background.

Soil Transmitted Helminths (STH) and other Neglected Tropical Diseases continue to promote poverty and frustrate the achievement of the health-related Sustainable Development Goals in endemic areas. To control the morbidity of STH, World Health Organization (WHO) recommends preventive chemotherapy for at risk populations. Several studies have accessed the impacts of this recommendation with conflicting results. This study was designed to evaluate the impact of preventive mass albendazole chemotherapy on changes in growth indices and school absenteeism.

Materials and methods

Albendazole tablets were administered by school teachers to 957children in the seven primary schools within a community in Southern Nigeria after data and stool sample collection. Follow up data were collected 6 months later for impact assessment. Ponderal growth retardation was defined as BMI under 5-percentile and longitudinal growth retardation as height for age under 5-percentile using the WHO classification scale.

Results

Helminth infection was found in 373 (39%) of the pupils before the intervention with *Ascaris lumbricoides* (n=247; 25.8%) and hookworm (n=89; 9.3%) being most prevalent. At enrolment 19.6% of children with and 11.8% without helminth infections had BMI below the 5-percentile. These figures were reduced to 9.2% and 8.8% after the intervention respectively. No effect of deworming was seen on longitudinal growth. The number of children with >25% absenteeism rate in the uninfected and infected group of children decreased by 7.2% and 13.9% respectively.

Discussion.

Even though a control group was missing in this study (for ethical reasons) the difference in response to deworming between infected and uninfected children strongly support an effect of deworming on growth and school absenteeism. The intervention could be administered by school teachers, thus allowing integration of the programme into existing structures.

Keywords=Helminthes, Body Mass Index, Absenteeism, Mass Drug Administration, Preventive Chemotherapy.

Background

Despite the evidence indicating that school based mass deworming is one of the cheapest ways to secure the future of children in developing countries, sustainability of the program as well as effective mass coverage continue to hinder the control of STH.[1]

Chronic infections with intestinal helminths are important health factors that may influence school performance and reduce social competence and regular school attendance. Studies in the United States of America (USA) have shown that worm infections are associated with lower literacy levels by 13% and lower earnings later in life by 43% [2]. The total lost years of schooling due to worm associated absenteeism, mostly in developing countries, amounts to over 200 million years, countries. [3] Helminth related average IQ loss in poor countries is also estimated as 3.75 points per worm infection.[4] Evidence has shown that improved health status associated with mass deworming leads to improved educational performance, increased productivity, life expectancy and decreased expenditure on health care [5] According to the WHO, more than 280 million children that are in need of deworming live in the 42 countries of the African Region, and more than 40% of these are from three countries where Nigeria leads with outstanding 21% [6]. Deworming coverage rate was higher in the countries of the American (46%) and South-East Asian (39%) regions compared to the African Region (32%). Though Nigeria achieved national deworming coverage of 22% in 2006, this dropped to 13% in 2008.[6]

School based mass deworming programs have traditionally depended on donor funds, since most of the countries where STH is endemic are poor. Programs are usually suspended or abandoned when donor funds run out. Surprisingly, most of the money required for the exercise is invested in staff training and organizing the logistics. For instance data from the Partnership for Child Development[7]showed that the cost of school-based deworming, including training and logistics was estimated to be around 50 US cent per child per year while the actual cost of the deworming tablet was 3 US cent per 400mg dose of albendazole per child [8.] The implication of this is that limiting or eliminating the cost of training and logistics will free more funds for drug procurement and enhance the sustainability of mass deworming programs. Another cause of setback in sustainability of mass deworming is the time factor. More time is often needed for cascade training of different category of staff than time needed to actually administer the tablets. [9] For example the Kenyan mass deworming program of 2008 was postponed twice because real and perceived logistical obstacles. [10]

It is therefore expedient to find cost effective and time saving ways that circumvent cost of training and reduced bureaucracy. We therefore decided to evaluate the effectiveness of a mass deworming program involving only school teachers without formal training. Drugs were distributed through the local clinic, and the study team was only present once at the start and once at the end of the project in order to minimize their influence on the study outcome.

Materials and methods

Study site and population

This study was carried out at Ilero town in Oyo State, South-West of Nigeria with tropical climate. Farming is the predominant occupation in this community. Tap water is also not available and the major potable water source is public wells, while defecating in the bush is the major means of sewage disposal. [11] The town has 7 public primary schools with a total population of about 1442 children. The pupils were aged 2 to 16 years consisting of pre-school and school aged children. The same children were studied for several other parameters,

published elsewhere, including helminth-malaria co-infections[12] and carrier status of diarrhoeagenic protozoa. [11]

Preliminary interviews with the parents and the school authority showed that mass deworming had never been carried out in this community before.

Study design

The study was designed as an effectiveness study with minimal influence and presence of the research team, which consisted of staff from the local clinic assisted by the first author. Thus study visits were restricted to a short interview, anthropometric measurements, and collection of a stool sample before the intervention and a single visit with anthropometric measurements and data collection 6 months after the intervention. The study was carried out in the mid of the school year between April and Sep 2013.The aim of the study was explained to the children, their parents or guardians and their teachers. The children were assisted to fill questionnaire forms in order to obtain their socio-demographic information after written informed consent was obtained from parents/guardians. The study was approved by the research and ethic committee of Federal teaching hospital, Abakaliki. The height in centimeter and weight in kilogram of each child was measured using digital weighing scale and wall mounted tape ruler respectively. The school attendance register was checked to record the number of days that each child was absent during the school term. School absenteeism was evaluated in the 100 days the school was open prior to the intervention and 100 days after the intervention. Data from the primary 6 pupils were not included in analysis because they were not available for follow up, having graduated before the revisit period.

Stool specimen collection

The children were given wide mouth, screw cap plastic bottles with clear instruction on how to transfer feces into it in the school premises. Children that were deemed too young were assisted with trained personnel in specimen collection.

The stool samples were divided in two parts immediately after collection, one part was preserved with equal volume of 5% formalin for microscopy while the other part was preserved with absolute methanol for PCR studies. The specimen was thoroughly mixed with the preservatives using applicator stick to ensure good preservation.

Drug administration

Teachers were asked to administer 400 mg of chewable albendazole tablets to the pupils irrespective of their age at a cost of about 5 US cents per child.

The teachers received no formal training regarding the deworming program. The process was explained to them a week before the program and they were encouraged to use their social contacts to sensitize the community to the exercise. Members of the research team only stood by as observers on the day of the actual deworming

Laboratory analysis

Formalin preserved stool samples were examined by direct microscopy for helminth ova and protozoan cysts by 2 independent microscopists and any discrepancy was resolved by having a third opinion. Methanol-preserved stool was frozen and transported to Copenhagen for molecular diagnosis of Giardiasis by PCR as described previously [11]

Data analysis

The data of weight, height, age and sex were used to calculate weight for age, Body Mass Index (BMI) and height for age using WHO growth tables and software [13]. Children were classified as reduced ponderal growth when BMI was less than 5 percentile (% ile) according to the WHO classification scale, those with BMI between 5 to 85 % ile were regarded as normal weight while those with BMI above 85%ile were classified as overweight[13]. Similarly, height for age was used as indicator for linear growth. The number of days of absenteeism before and after intervention were grouped into ranges from those who were absent for less than 5% through those who were absent for more than 25%. Because it was considered unethical to randomize children to treatment or placebo we used a different approach to document the effect of deworming. Thus, we divided the children into two arms for the data analysis, one consisting of children with detectable helminths and the other without helminths prior to treatment.

Association between presence or absence of helminth egg in stool and weight-for-age before and after deworming was initially calculated using McNemar's chi square test calculator (GraphPad Software, Inc. 2015 edition),[14] and p-values less than 0.05 were considered significant. Subsequently, a linear mixed effects (LMM) model was used to test for an association between parasite microscopy status (i.e. infection by at least one species of helminth egg) and growth indicators. More specifically, the z-scores were regressed on a parasite status indicator, an indicator marking whether the value was obtained at follow-up and an interaction term between the two; a random intercept variable was used to account for repeated sampling of the same individual. The models were fit using the *lmer* package (package lme4, version 1.0-6)[15] For example; the following function call was used for weight: Lmer (weight_z ~ follow_up * parasite_mic + (1|id), data = data, REML = F). Likelihood ratio tests were used to assess statistical significance with null distributions obtained using parametric bootstrap facilities provided in the pbkr test package. The same approach was used for modeling school absence time except that a generalized linear mixed effects model (GLMM) with a Poisson distribution and log link-function (also from *lmer*) was used in place of the LMM used for the continuous variables. P-values <0.05 were considered significant.

Results

We observed that the teachers complied reasonably with the deworming instructions given, and that the community was effectively sensitized, judging by the consent and high acceptance rate from the pupils. We screened a total of 957 children (473 boys and 484 girls) with a median age of 8 years and in the range 2-16 years (Figure 1). Three hundred and seventy three (39%) of the investigated children were infected with at least one helminth species (Table 1). *Ascaris lumbricoides* (25.8%) and Hookworm (9.3%) were the commonest followed by mixed infection with the two parasites (1.8%).

The helminth positive children were almost twice as likely as the helminth negative children to be underweight prior to deworming (19.6% vs. 11.8%, Table 2, p=0.0001). Six months after deworming the prevalence of low weight-for-age had dropped to approximately 9% in both groups of children and the difference between helminth status prior to deworming had disappeared (Table 2, p=0.85).

In the helminth positive group, McNemar's test showed that changes in BMI was significantly associated with deworming (p<0.0001, chi square=20.833 with 1 degree of freedom, odds ratio=14.000), while a similar association was also observed in the helminth negative group of pupils (p=0.0027, chi square=9.031 with 1 degree of freedom, odds ratio=3.571)

To confirm the test whether positive helminth status prior to deworming was associated with weight improvements at follow-up, a LRT was used to compare a model considering only age, sex, time (i.e. follow-up or baseline) and parasite microscopy status with a model also including an interaction term between follow-up and parasite microscopy status. As shown in Figure 2 there was a significant overall effect of deworming on weight-for-age and BMI ($p< 0.05$ and $p< 0.05$, respectively) and this effect was most pronounced for children with detectable helminth eggs in their stool (interaction between helminth and follow up $p<0.05$). In contrast, deworming had no effect on height-for-age in any of the groups ($p>0.05$). For model estimates, see supplemental figure S1. Regarding the effect of deworming on absenteeism, we observed a reduction in the number of children with large percentage of absenteeism after deworming. The median number of days of absenteeism before and after deworming was 14 days (Range 0-94) and 9 days (Range 0-62), respectively.

To test whether positive parasite microscopy status was associated with improvements in school absenteeism at follow-up, two GLM models corresponding to the LMM described above were fitted to the absenteeism outcomes and a LRT used to test for significance of the interaction effect. The results are summarized in figure 2D and showed a significant effect of deworming with an interaction between helminth status ($p=0.001$). Although the effect of deworming on weight and school absenteeism was more pronounced in the children with detectable helminth eggs in stool prior to the intervention, there was also a significant effect in helminth negative children. We speculated that this could be explained by an effect of albendazole on giardiasis and thus went on to test a random subset of the stool samples for *Giardia* using PCR. Giardia DNA was detected in 64 of 180 stool samples and tended to be more common in underweight children. However, a LMM analysis did not show any association between positive *Giardia* PCR and any of the parameters (data not shown)

Discussion

The high prevalence of helminth infection seen in this community (39%) is a likely consequence of lack of potable water combined with the poor sewage disposal methods in this community.[11] A survey of four primary schools in Kenya showed a similar prevalence ranging from 31% to 48.9%, [16] while 58.3% prevalence was also reported among Ethiopian children[17] in communities with similar challenges of water, sanitation and hygiene. A higher prevalence (68.2%) was once found in a similar study in Nigeria by Dada-Adegbola *et al*[18] while Kirwan et al reported 50% prevalence in a similar Nigerian study. [19]

Factors responsible for growth retardation of children in developing countries are complex and multifactorial. Apart from helminth infection, other factors attributable include undernutrition, low family income and large family size[20]. This may explain why some studies found little or no effect of deworming intervention on weight gain.[21, 22] However; other studies found deworming to significantly improve weigh gain. The study of Ethiopian children [17] also found an association between helminth infection and underweight among the school children, and the weight-for-age z-scores of the children significantly increased four weeks after treatment for helminth infection, with a single dose of albendazole. A clinical trial in India also showed that a single albendazole treatment of helminth infected school children lead to a significant weight gain.[23] Our attempt to find out possible causal association between the observed weight changes in the helminth negative children and Giardia infection yielded no significant result. We thus speculated that this may be part of complex multifactorial factors responsible for BMI that could not be controlled for in this study design. Our data thus suggested a causal relationship between intestinal helminth infection and ponderal growth

changes in the study population. Lack of effect of our deworming on height of the children was not surprising. A result of 10 longitudinal studies which compare growth of children infected with *Ascaris* with antihelmintic treatment showed that it was less likely to associate linear growth to deworming than ponderal growth. [24] This may be because stunting results from chronic undernutrition which retard linear growth unlike ponderal growth deficit which occur over a shorter period of time [23] and hence changes are also observable over a shorter period of time. Similar to our study, a deworming exercise in Malaysia did not find significant height gain even when the children were followed up for 2 years [21]. Contrary to our findings, Awasthi *et al* in India showed that deworming caused improved height gain compared to children in the control study, but the treatment was repeated every 6 months for 2 years, as opposed to our study [23]. Interestingly, Koroma et al also found significant changes in height for age of Sierra Leonean children after 6 months of albendazole intervention contrary to our result[25]. Our finding showed a surprising strong effect of deworming on school attendance. Studies evaluating the impact of deworming on school attendance have generated conflicting reports. For instance, the Kenyan study by Muguel and Kramin [26] showed that deworming improved school attendance. This was supported by another study in Jamaica which also showed that helminth infection was associated with poor school attendance and deworming led to a significant improvement in absenteeism 6 months after treatment, a benefit that was more pronounced in infected children who were also stunted. [27] On the other hand, Davey et al [28] recently concluded that benefit of deworming on school attendance remains controversial.

It is not unlikely that the initiation of our study could have sensitized the teachers to improve on school attendance record keeping which could be a confounding factor. Also as a predominantly farming community, seasonal variation in farming activities such as planting, weeding and harvesting could also interfere with school attendance, which we may not be able to account for. We therefore like to suggest that the deworming effect on absenteeism observed in our study needs cautious interpretation and should be subjected to further investigation that will take into consideration all possible variables.

By limiting our role during the deworming exercise to that of observership and data collection at few time points, the results of our study showed that school based mass deworming could be effectively carried out by the teachers without investing most of the donor funds in formal training. This in turn could free more funds for drug procurement and reduce donor fatigue which is a major obstacle to sustainability of mass deworming programs. Minimizing the interference of the research team also allows us to have a more realistic picture of the real life situation and the effectiveness of the program as opposed to efficacy studies, which require more control and follow up by the investigation team.

Studies that have employed community integrated approach in deworming programs reported that the approach is effective and feasible. [29-31] Also a study about community perception of school based deworming in Turkey showed that 87.4 % of the parents were aware of school health programs and 99% of them approved of teachers' role in providing health education and administering deworming tablets to pupils.[32] The same study also showed that 85% of the parents were willing to pay for the exercise, and this may support the enthusiasm that greeted the end of our study visit during which virtually all the schools presented gifts to our research team. Our exercise cost about 5 US cent per 400mg dose of albendazole, indicating that about 2000 children could be effectively dewormed with just 100 USD. This suggested that deworming could be done effectively at minor expenses through interaction between public health promoters and the community. However, monitoring of large scale efforts of integrating deworming in existing facilities is important in order to detect obstacles to this approach.

Our data support the usefulness of regular deworming and indicate that an effect will last at least 6 months. It seems possible to motivate schools to support the program at minimal cost. Further studies are needed to evaluate and monitor rolling out of this program.

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Table 1.Helminths detected in children prior to deworming

Helminth	N (%)
Ascaris	247 (25.8)
Hookworm	89 (9.3)
Ascaris+Hookworm	17 (1.8)
Trichuristrichuria	6 (0.6)
Enterobius	5 (0.5)
0ther	9 (0.9)
Negative	584 (60.8)
Total	957

Table 2. Prevalence of abnormal BMI before and after deworming by helminth carrier status. For definitions, see text.

		BMI group, N (%)			Total
		Underweight	Normal weight	Overweight	
Before deworming[1]	Helminth Negative	69 (11.8)	479(82.2)	35(6.0)	583 (100)[2]
	Helminth Positive	73(19.6)	288(77.2)	12(3.2)	373 (100)
	Total[2]	142	767	47	956
After deworming	Helminth Negative	51 (8.8)	475(81.8)	55(9.5)	581 (100)
	Helminth Positive	34(9.2)	296(80.4)	38(10.3)	368(100)
	Total[3]	85	771	93	949(100)[4]

[1]Significant difference between helminth negative and helminth positive children, p=0.0001
[2]Anthropometric measurements missing from 1 child
[3]Helminth status assessed prior to deworming
[4]Follow up was missing for 7 children

Figure legends

Figure 1: Age-distribution of study population prior to deworming.

Figure 2: Mixed effects parameter models for growth parameters and school absenteeism before and 6 months after deworming of 957 Nigerian school children divided by presence or absence of intestinal helminth eggs prior to the intervention. Growth parameters are calculated as z-scores, where 6 months increase in age has been added to the age of the children between study entry and follow up. For calculations, see text. For model estimates of the weight calculation, see Table 3. Other model estimates are summarized in the text. (Clockwise) A. Weight for age, B. Height for age, C. BMI for age, D. School absenteeism. Overall effect of deworming at follow up: - A. $p < 0.05$, B. $p > 0.05$ (not significant), C. $p<0.05$, D. $p<0.05$. Interaction between helminth status and follow up: - A. $p < 0.05$, B. $p>0.05$ (not significant), C. $p < 0.05$, D. $p < 0.05$.

Figure 3: Distribution of school absenteeism during 100 days before deworming and 100 days before 6 month follow up. A= helminth negative and B=helminth positive prior to the intervention.

Figure S1: Run result of LRT models of effect of deworming on anthropometric parameters.

Figure 1

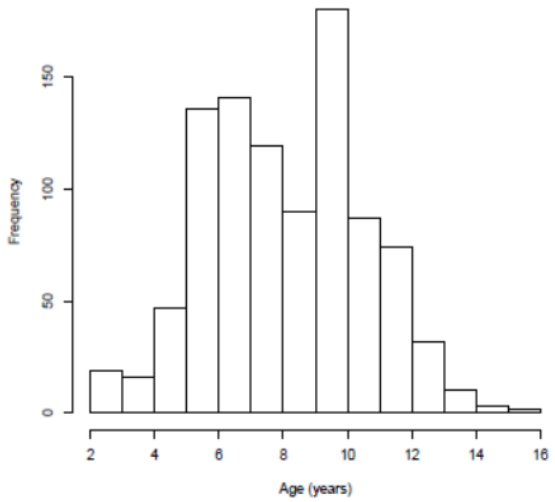

Figure 2

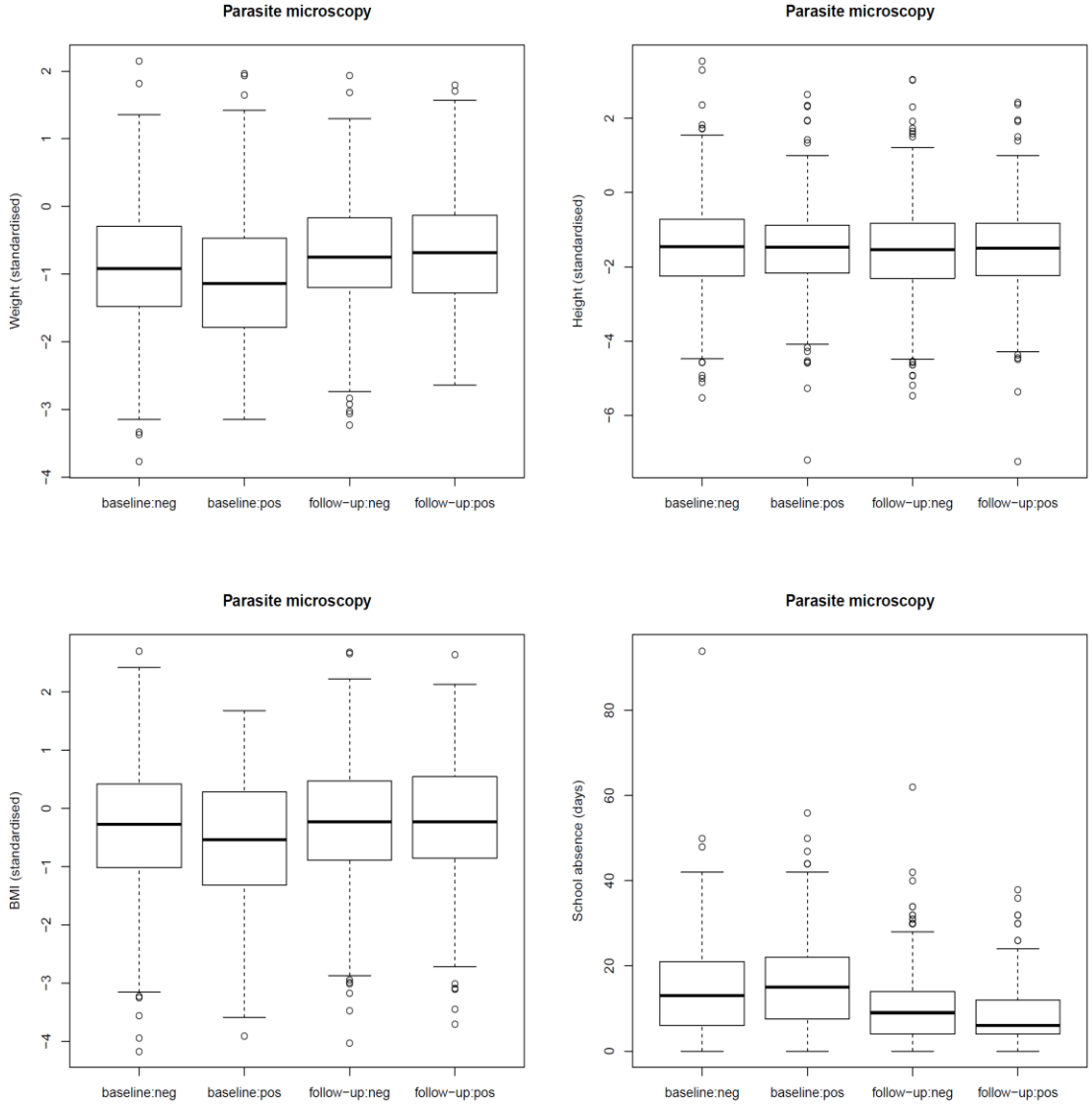
Parasite microscopy
Weight (standardised)
baseline:neg
baseline:pos
follow−up:neg
follow−up:pos
Parasite microscopy
Height (standardised)
baseline:neg
baseline:pos
follow−up:neg
follow−up:pos
Parasite microscopy
BMI (standardised)
baseline:neg
baseline:pos
follow−up:neg
follow−up:pos
Parasite microscopy
School absence (days)
baseline:neg
baseline:pos
follow−up:neg
follow−up:pos

Figure 3

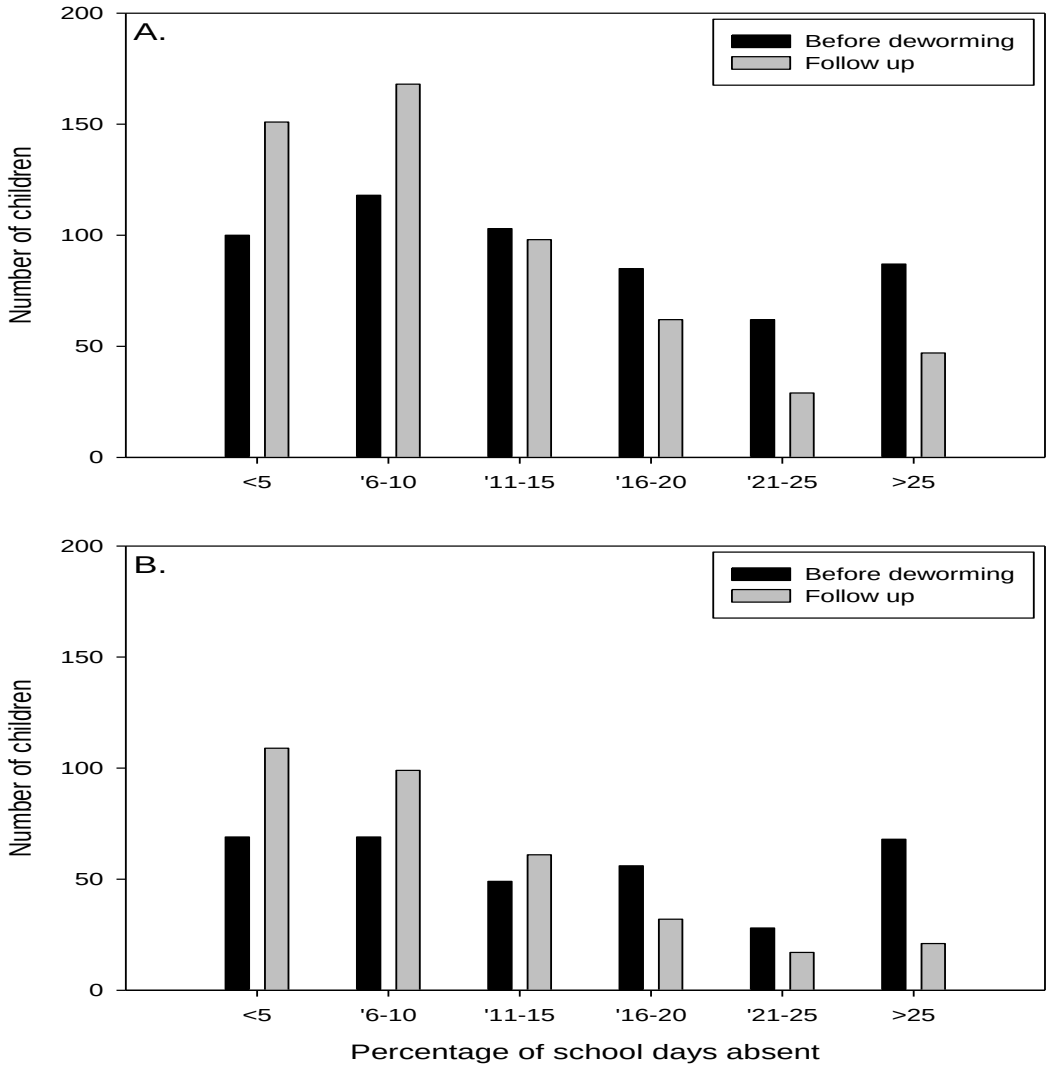
A.
Before deworming
Follow up
Number of children
200
150
100
50
0
<5
'6-10
'11-15
'16-20
'21-25
>25
B.
Before deworming
Follow up
Number of children
200
150
100
50
0
<5
'6-10
'11-15
'16-20
'21-25
>25
Percentage of school days absent

Figure S1

```
> PBmodcomp(wfa_model_parasite_interaction, wfa_model_parasite)
Parametric bootstrap test; time: 33.55 sec; samples: 1000 extremes: 0;
large : weight_z ~ follow_up * parasite_mic + (1 | id)
small : weight_z ~ follow_up + parasite_mic + (1 | id)
         stat df   p.value
PBtest 93.448     0.000999 ***
---
```

The estimated parameters of the model are:

```
Fixed effects:
                                Estimate Std. Error t value
(Intercept)                    -0.115208   0.402420  -0.286
age                            -0.007401   0.004018  -1.842
sex                             0.189089   0.243388   0.777
follow_upTRUE                   0.135182   0.016476   8.205
parasite_micTRUE               -0.236835   0.067250  -3.522
age:sex                        -0.002618   0.002441  -1.072
follow_upTRUE:parasite_micTRUE  0.210434   0.021240   9.907
```

3.0 Summary

The high prevalence of intestinal helminths reported from our study site as well as the large number of children that still dispose of feces in the bush implies that attention should be paid to improved STH control. We were not able to show particular risk factors in this population, but it is well established that such risk factors include reduced access to safe water and sanitation and low levels of health education. Our findings thus suggest that despite some differences in these factors in the population, the overall level of sanitation and health education is so low that we could not demonstrate an effect of these. This calls for urgent improvements in these factors. The improvement in BMI among the pupils after 6 months of mass deworming further confirmed the beneficial effect of school based albendazole PC. The result of this study is in agreement with the early impact assessment of mass deworming in Uganda which showed that children in the treatment group had significant increase in weight gain compared to controls.[56] Single dose of albendazole treatment of school children was also associated with demonstrable growth improvement in Kenya[57] in a research carried out by Schuurman *et al*. Our study design also showed that school based mass deworming could be effectively carried out by the teachers without investing most of the donor funds in formal training. This in turn could free more funds for drug procurement and reduces donor fatigue which is a major obstacle to sustainability of mass deworming programs.

The observed negative association between malaria and helminths among the pupils suggest that mass deworming exercises might also put the younger children in an increased risk of developing clinical malaria. Hence it may be necessary to combine STH control programs with intermittent preventive treatment of malaria in children (IPTc) or other interventions aimed at reducing malaria risk. Our study design did allow us to directly monitor deworming effect on malaria, so we would recommend that future studies are done as intervention trials

so as to scrutinize the effect mass deworming on malaria risk and other health consequences in groups, with or without simultaneous administration of IPTc.

Regarding the molecular diagnosis of protozoan parasites, the absence of *E. histolytica* in our study implies that E. histolytica colonization is not common in this population. Our finding was contrary to previous studies which reported high prevalence of *E. histolytica*, probably because the diagnostic methods employed previously were less specific and could not distinguish it from the non-pathogenic *E. dispar* which we found to be highly prevalence in our study. Our result has thrown into question the commonly accepted figure of 500 million *E. histolytica* infections worldwide, suggesting that the true global prevalence may have been largely over-estimated. It is recommended that researchers should use real-time PCR to re-evaluate the global epidemiology of this particular pathogen so as to be able to better match empirical treatment with diarrhea-causing microorganisms in resource limited countries, especially within the context of IMCI.[55] One limitation of our study was the fact that our study subjects were not evaluated for viral and bacterial pathogens which are also very important diarrhea agents in this part of the world. Such evaluation is beyond the scope of this work and will overstretch our available resources. It is therefore recommended that further studies will be needed so as to complement our result and produce a full overview of all diarrhea agents in this area.

The high prevalence of Giardia from our real time-PCR result is in keeping with the high sensitivity of this diagnostic tool. The high sensitivity implies that carrier states could not be distinguished from clinically relevant infections and as such, it could be of limited values for routine diagnosis of Giadiasis. This was also confirmed by the high Ct of the microscopy negative samples. High sensitivity of Giardia real-time PCR was also demonstrated in a study in Netherlands when it was compared with microscopy and ELISA. The Netherlands study concluded that microscopy should remain the primary diagnostic tool for identifying *G.*

lamblia in clinical cases despite its lower sensitivity because real-time PCR had the lowest predictive value.[57] The major advantage of the high sensitivity on the other hand is that, it is a better epidemiologic tool when compared to other diagnostic alternatives.[57]

Publication 1

Abstract: The relationship between intestinal helminth infection and susceptibility to malaria remains unclear. We studied the relationship between these infections. Seven schools in Ilero, Nigeria referred all pupils with febrile illness to our study center for free malaria treatment during a 3-month study period. At the end, all pupils submitted a stool sample for microscopic investigation for helminth eggs. We used an unmatched case-control design to calculate the odds ratios for helminth infection in children with at least one attack of malaria (cases) and children with no malaria episodes during the study (controls). We recorded 115 malaria cases in 82 of 354 (23.2%), 16 of 736 (2.2%), and 17 of 348 (4.7%) children ages ≤ 5, 6–10, and 11–15 years old, respectively (P = 0.001). Helminth infection rate in cases was 21 of 115 (18.3%) compared with 456 of 1,327 (34.4%) in controls. Weighted odds ratio stratified by age group for helminth infection in cases versus controls was 0.50 (95% confidence interval = 0.2–0.84, P < 0.01). Ascaris and hookworm were the most common helminths detected, with prevalence rates of 14 (12.2%) and 6 (5.2%) among cases compared with 333 (25.1%) and 132 (10.0%) in controls, respectively (P = 0.001). The negative association between helminth infection and malaria may be of importance in the design of deworming programs.

Publication II

Abstract: Diarrhea remains the second largest killer of children worldwide, and Nigeria ranks number two on the list of global deaths attributable to diarrhea. Meanwhile, prevalence studies on potentially diarrheagenic protozoa in asymptomatic carriers using molecular detection methods remain scarce in Sub-Saharan countries. In order to overcome sensitivity

issues related to microscopic detection and identification of cysts in stool concentrates, real-time PCR was used to analyze genomic DNAs extracted from stool samples from 199 healthy school children for *E. histolytica, E. dispar, Giardia intestinalis,* and *Cryptosporidium*. Questionnaires were administered for epidemiological data collection. *E. histolytica* was not detected in any of the samples, whereas *Giardia* (37.2%), *E. dispar* (18.6%), and *Cryptosporidium* (1%) were found. Most of the children sourced their drinking water from community wells (91%), while the majority disposed of feces in the bush (81.9%).Our study is the first to use RT-PCR to evaluate the epidemiology of *E. histolytica, Giardia* and *Cryptosporidium* in Nigeria where previous studies using traditional diagnostic techniques have suggested higher and lower carriage rates of *E. histolytica* and *Giardia,* respectively. It is also the first study to accurately identify the prevalence of common potentially diarrheagenic protozoa in asymptomatic carriers in Sub-Saharan Africa.

Paper III (In preparation)

Abstract: To control the morbidity of intestinal helminths, World Health Organization recommends Preventive Chemotherapy (PC) for at risk populations. But PC sustainability is hampered by limited donor funds in most settings. It is therefore necessary for find cost effective means of sustaining the program. This study was designed to evaluate effectiveness of mass deworming using school teachers without formal training as a means of by-passing the huge cost of training so that more funds could be made available for deworming tablets.

Albendazole tablets were administered by class teachers without previous training to 957children in the seven primary schools within the community after data and stool sample collection. Follow up data were collected 6 months later for impact assessment.

Helminth infection was found in 373 (39%) of the pupils before the intervention, with *Ascaris lumbricoides* (n=247; 25.8%) and hookworm (n=89; 9.3%) being most prevalent. At

enrolment 19.6% of children with and 11.8% without helminth infections were low weight-for-age. These figures were reduced to 9.2% and 8.8% after the intervention respectively. But there was no significant effect of deworming on height-for-age in either category of children. Intervention reduced the number of low weight-for-age infected and uninfected pupils by 10.4% and 3.0% respectively. The number of children with >25% absenteeism rate in the uninfected and infected group of children decreased by 7.2% and 13.9% respectively.

By limiting our role during the deworming exercise to that of observership and data collection, our study showed that mass deworming could be effectively carried out by the teachers without investing most of the donor funds in formal training. Our data also support the usefulness of regular deworming and indicate that an effect will last at least 6 months.

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Annexes

Annex I. Declaration of Authorship

I hereby declare that the work presented in this dissertation has been designed and performed

independently, without help from others and without other materials than stated in the text. To the best of my knowledge and belief, thoughts and ideas from other people and colleagues that have been adopted directly or indirectly in this dissertation were specifically indicated and acknowledged in any case. I confirm that others did not either directly or indirectly receive any payment in kind for any work related to the content of this dissertation. This dissertation has never been submitted before for the award of any other degree or during any kind of examination procedure in any other institution.

Signature..... Leipzig……..

Efunshile Akinwale Michael Place

Annex II. CURRICULUM VITAE

EFUNSHILE AKINWALE MICHAEL

Name: Efunshile Akinmale Michael

Date of Birth: August 8th, 1967

Place of Birth: Abeokuta, Nigeria

Nationality: Nigerian

Language: Yoruba, English and German

E-mail: drefunshile@yahoo.com

Educational Background

Primary/Secondary school

1986-1980: Methodist Primary School, Ogbe, Abeokuta.

1980-1985: Igbore High School, Abeokuta, Nigeria

University / Collage

Year Degree Field of study University/Collage

Year	Qualification	Field of study	Awarding Institution
2011-2015	Doctorate	Medical Parasitology, Epidemiology of Infectious Diseases.	University of Leipzig, Germany
2005-2012	FMC.path	Clinical Microbiology	National Postgraduate Medical College of Nigeria
2004-2005	M.Sc.	Medical Parasitology	University of Lagos, Nigeria
1994-1999	MB.BS	Medicine and Surgery	University of Lagos, Nigeria
1988-1991	BSc	Microbiology	University of Ibadan, Nigeria
1985-1987	A' Level	Basic Sciences	Ogun State Poly/WAEC, Nigeria

Work experience:

2000-2001: Medical House Office, Nigerian Army Reference Hospital, Yaba, Lagos, Nigeria

2002-2005: Resident Doctor, Medical Microbiology Department, Olabisi Onabanjo, University Teaching Hospital, Nigeria.

2005-2009: Lecturer 1, Medical Microbiology Department, College of Medicine, Olabisi, Onabanjo University, Nigeria

2010-2013: Lectuter 1, Medical Microbiology Department, College of Medicine, Ebonyi State University, Nigeria

2013- Till date: Senior Lecturer, Medical Microbiology Department, College of Medicine, Ebonyi State University, Nigeria.

Awards and Grants

1) 1991: Faculty of Science award, University of Ibadan.
2) 2010: German Academic Exchange Service (DAAD), Short Research Stay Fellowship: Drug resistant malaria study, Otto Von Guericke University, Magdeburg, Germany.
3) 2011: German Academic Exchange Service (DAAD). PhD Fellowship, Leipzig University
4) 2013: ASM Travel Grant to USA Conference, American Society for Microbiology.

Efunshile Akinwale Michael……..

Leipzig……

Annex III. List of Publications

1. **Efunshile AM**, Temitope Olawale, Christen Rune Stensvold, Jørgen A. L. Kurtzhals, and Brigitte König Epidemiological Study of the Association Between Malaria and Helminth Infections in Nigeria. Am J Trop Med Hyg 2015; 92:578–582

2. **Efunshile AM**, Ngwu BAF, Kurtzhals JAL, Sahar S, König B, Stensvold CR. Molecular detection of the carriage rate of four intestinal protozoa with real-time PCR—possible over-diagnosis of *Entamoeba histolytica* in Nigeria. Am J Trop Med Hyg 2015; 93:257-262
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7. Cytomegalovirus and HIV Co-Infection Among. Patients Accessing Care in a Tertiary Care Centre in Nigeria. Fowotade A, Agbede O, Salami A, Fayemiwo A, **Efunshile A**, 2013. Sex Transm Infect 89:A1–A428

8. **Efunshile AM**, Fowotade A, Makanjuola OB, Oyediran E I, Olusanya OO, Koenig B, 2013. Antimalarial use and the associated factors in rural Nigeria following implementation of Affordable medicines facility-malaria (AMFM) price subsidy. Afr J Cln Exper Microbiol 14: 88-94.
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10. Adefioye OA, **Efunshile A** , Ojurongbe O, Akindele AA, Adewuyi IK, Oloyede S, Adedokun SA and Adeyeba AO, 2011. Intestinal Helminthiasis among School Children in Ilie, Osun State, Southwest, Nigeria.*Sierra Leone J Biomed Res* 3:36-42.
11. **Efunshile M** , Amoo AOJ, Akintunde GB, Ojelekan OD, König W, König B, 2011. Use and Effects of Malaria Control Measures in Pregnancy in Lagos, Nigeria.*Korean J Parasitol 49:365-371*.
12. Adedeji SO, **Efunshile AM**, Oyinloye JM, 2010. The Prevalence of Asymptomatic Urinary Tract Infection and Antibiotic Resistance Pattern of Isolates in Apparently Healthy Secondary School Students in Sahamu Local Government Area, Ogun State, Nigeria. Int J of Biomed sc2:38-44.
13. Abudu EK, Akinde OR, Oyebadejo TO, **Efunshile AM**, Musa OA, Banjo AA, 2010. Histopathological study of soft tissue malignancies in a teaching hospital, Sagamu, South-West Nigeria. *Nig Q J Hosp Med* 20:42-45
14. **Efunshile AM**, Oduyebo OO, Oyibo WA, Ogunsola FT, Fatungase AO, Osinupebi AO. Knowledge, attitude and practices of the trainee seafarers to HIV/AIDS and STIs at Apapa seaport, Lagos. Afr J Cln Exper Microbiol 2007. 8:94-100.
15. **Efunshile M**, Runsewe-Abiodun TB. Ghebremedhin B, König B, König W. Prevalence of the molecular markers of chloroquine resistance (PFCRT 76) five years

after the withdrawal of the drug as first line antimalarial, a cross sectional study. SAJCH 2011.5:39-42.

16. Abudu EK, Oyebadejo TO, Akinde OR, **Efunshile AM**, Musa OA, Awolola NA, Banjo AAF . Vasoformative Neoplasms in a Teaching Hospital, Sagamu, Ogun State: A Histopathological Review. Nig J of Health 2009. 8
17. Ogunledun A, Deji-Agboola AM, **Efunshile AM**, Mutiu WB, Banjo TA, Adedeji SO, Igile GO. *In-vitro* Antimicrobial Efficacy of Carex Powerful Antiseptic Liquid Nig J Hlt and Biomed Sci 2008. 7(2)

Annex IV. Papers Presented at Scientific Conferences

1) Effect of mass deworming on changes in body mass index and school absenteeism in pupils in a semi-rural area of Nigeria. **ICAAC 2014**. 54th Interscience Conference on Antimicrobial Agents and Chemotherapy. September 5 -9, 2014 | Washington, DC. Walter E. Washington Convention Center. USA
2) Epidemiologic study of the Relationship between Malaria and Helminth Infections in Ilero town; Nigeria. **ICAAC 2014**. 54th Interscience Conference on Antimicrobial Agents and Chemotherapy. September 5 -9, 2014 | Washington, DC. Walter E. Washington Convention Center. USA
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